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Reaction-Intermediate Analogues: Design, Syntheses, and Inhibitory Studies With Carnitine Acetyltransferase.

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REACTION-INTERMEDIATE ANALOGUES: DESIGN, SYNTHESES, AND INHIBITORY STUDIES WITH CARNITINE ACETYLTRANSFERASE

A Dissertation

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To my parents, brothers, and sister for their encouragement.

To my wife, Xia Jin, for her love, patience, and support.

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LIST OF ABBREVIATIONS

[α]	specific rotation
calcd	calculated
CAT	carnitine acetyltransferase
CoA	coenzyme A
concd	concentrated
COT	carnitine octanoyltransferase
CPT	carnitine palmitoyltransferase
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMSO	dimethyl sulfoxide
DNB	3,5-dinitrobenzoyl
ee	enantiomeric excess
FAB	fast atom bombardment (in mass spectrometry)
m-CPBA	*m*-chloroperoxybenzoic acid
MMPP	monoperoxyphthalic acid, magnesium salt hexahydrate
Ms	methanesulfonyl (mesyl)
MTPA-Cl	(*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride
PNB	4-nitrobenzoyl
satd	saturated
THF	tetrahydrofuran

ABSTRACT

Carnitine acetyltransferase (CAT) catalyzes the reversible transfer of short chain (less than six carbons in length) acyl groups between carnitine and coenzyme A (CoA). This reaction likely modulates the reserves of free CoA and acetyl-CoA in each organelle and membrane in ways specific to the local demands. To probe the structures of the molecular interactions between carnitine and the active site in CAT, we have designed and synthesized conformationally constrained reaction-intermediate analogues, which not only inhibit enzymatic activity, but also help reveal the topography of the active site.

The syntheses of (*2R,6R*)-, (*2S,6S*)-, (*2R,6S*)-, and (*2S,6R*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium are described. The single-crystal X-ray structures of these compounds are presented. These four stereoisomers are tested as specific inhibitors of CAT. The results confirm the hypothesis that there is two-point recognition by CAT for carnitine and acetyl-CoA is the third locus for chiral recognition. The results also strongly support the proposed mechanism for acetyl transfer between carnitine and CoA.

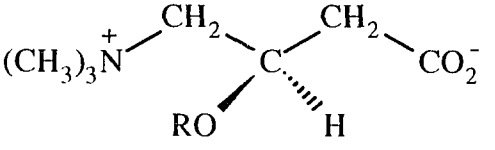
The syntheses of methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate and the resolution of 5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile are also described.

CHAPTER I

INTRODUCTION

I.1. GENERAL

Carnitine (3-hydroxy-4-trimethylammoniobutanoate, see below), a natural constituent of higher organisms, is absolutely necessary for efficient



R = H; carnitine.

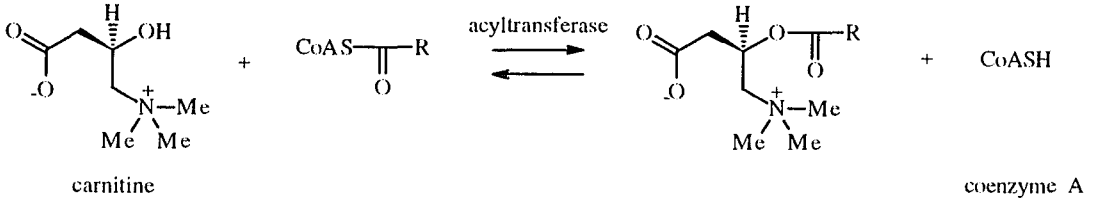
R = acyl; acylcarnitine.

metabolism of long-chain fatty acids.[1-5] Fatty acids are activated by forming acyl-coenzyme A (acyl-CoA) on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix. Long-chain acyl-CoA molecules cannot penetrate the inner mitochondrial membrane leading into the matrix. Acyl groups must be transferred to carnitine to form acylcarnitine, which can penetrate the membrane. Once inside the matrix, the acyl groups are transferred back to coenzyme A (CoA) and oxidation can then occur. The oxidation of fatty acids occurs also in peroxisomes.[3,4,6,7] The oxidation in peroxisomes is not complete, but terminates at acyl-CoA residues

of short- and medium-chain length.[3] These chain-shortened acyl-CoA residues are then esterified to carnitine and the resulting acylcarnitines are transported out of peroxisomes for further metabolism. A recent study[8] suggests that carnitine is involved in the metabolism of both short- and long-chain acyl-CoAs within lymphocytes and phagocytes. Carnitine may play a significant role in the metabolism of medium-chain fatty acids as well.[9] In addition, carnitine can buffer the acylation state of the CoA pool in ways specific to the local metabolic demands.[10]

Carnitine is essential for good health. Tissues with inadequate concentrations of carnitine exhibit severely impaired cellular energy metabolism.[1] A diverse collection of diseases are related to carnitine.[1,5] Since the discovery of the first case of human carnitine deficiency,[11] carnitine has been used for therapy.[12] Alzheimer's disease is the most common cause of dementia in developed countries and affects about 3-4 million Americans.[13] The progression of Alzheimer's disease can be significantly reduced by administration of acetylcarnitine.[13] Carnitine also plays an essential role in human nutrition.[5,14] Requirement for carnitine is increased by rapid growth (infancy, or the repletion of protein-calorie undernutrition), pregnancy, lactation, metabolic acidosis, certain drugs excreted in acidic form, renal dialysis and renal tubular disorders.[14]

Carnitine functions as an acyl carrier in fatty acid metabolism and acts as a buffer for the acyl-CoA/CoA ratio. The reversible acyl transfer between carnitine and CoA is catalyzed by carnitine acyltransferases



Scheme I.1

(Scheme I.1).[2-4,15] Therefore, both carnitine and carnitine acyltransferases play vital roles in fatty acid metabolism and are essential for good health. Carnitine acetyltransferase (CAT), carnitine octanoyltransferase (COT), and carnitine palmitoyltransferase (CPT) have been identified. They are classified on the basis of their subcellular localization and substrate specificity.[2] CAT, which is selective for short-chain acyl groups, is found in mitochondria, peroxisomes, and microsomes.[16] COT with specificity for medium-chain/long-chain acyl groups is found in peroxisomes and microsomes.[17-21] CPT, selective for long-chain acyl groups, is found on the mitochondrial outer membrane (CPT-I) and in the mitochondrial matrix (CPT-II).[17,18,22-27] CPT is also found in microsomes,[28] peroxisomes,[29,30] erythrocyte plasma membrane,[31] and sarcoplasmic reticulum.[32] Considerable overlap of acyl chain length selectivity occurs for COT and CPT. Our long-range goal is to elucidate the structures of the molecular interactions between carnitine and the active sites on these enzymes. Identifying these molecular interactions will lead to a better understanding of the physiological chemistry and, more specifically, of the regulation of the enzymes. This project will focus on CAT.

I.2. CARNITINE ACETYLTRANSFERASE

CAT is widely distributed in nature, occurring in different organisms from yeast to mammals.[2] Very high CAT activities are observed in rat heart, testis, and brown adipose tissue.[33] CAT is also uniformly distributed in the nervous system.[34,35]

CAT has been purified from different tissues and organelles, including pigeon breast muscle homogenate,[36-39] pig heart,[40] mouse liver peroxisomes,[20] yeast peroxisomes,[41] yeast mitochondria,[41] yeast cell-free extract,[42,43] rat liver mitochondria,[44,45] rat liver peroxisomes,[46] rat liver microsomes,[46] rat liver homogenate,[45] bovine heart,[37,47] bovine spermatozoa,[47] chick embryo liver,[30] and human liver.[48] In general, CATs from different sources have similar properties with molecular weight ranging from 51,000 to 75,000 and pH optimum between 7.2 and 8.0.[16] Most of the CATs show the highest activities with C_3 or C_4 acyl groups.[16]

The wide distribution of CAT indicates its important physiological functions. The role of CAT in buffering acetyl-CoA/CoA ratio in the mitochondrial matrix has been established.[10] In the mitochondrial matrix, free CoA is required for the function of the citric acid cycle, for β-oxidation, for the detoxification of organic acids, for the oxidative degradation of amino acids, and as a substrate for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Therefore, lack of free CoA can limit the mitochondrial capacity for energy production. CAT can modulate the acyl-CoA pools and facilitate formation of free CoA under conditions of accumulating acyl-CoA.

In human muscle tissue, an increase in acetylcarnitine occurs during endurance exercise,[49,50] indicating that, under the regulation of CAT, carnitine functions as an acceptor for an acetyl group to buffer excess acetyl-CoA formed from pyruvate decarboxylation and β-oxidation and release CoA for energy production. The modulation of the acyl-CoA pools may also be important in metabolic regulation in normal tissue,[2] protecting the tissue from build-up of toxic acyl-CoA[51-53] and providing a pathway for storing or excreting the acyl moieties if their normal metabolism is impaired.[2,53,54] Acetylcarnitine can serve as a reservoir of activated acetyl units and provide an immediate source of energy.[2,10] CAT found in endoplasmic reticulum and in peroxisomes can convert cytosolic acetylcarnitine into acetyl-CoA, which could be used for the synthesis of malonyl-CoA in the heart. Accumulation of acetyl-CoA in the cytoplasm would also serve to decrease the free CoA available for fatty acid activation.[55] CAT in each organelle or membrane can modulate the reserves of free CoA and acetyl-CoA in ways specific to the local demands.[10] The buffer role of CAT in general fits in well with the tissue distribution of the enzyme, which correlates with high metabolic activity.[56,57] Another important role for CAT is that it may shuttle short-chain acylcarnitines out of peroxisomes for tissues that chain-shorten long-chain fatty acids via peroxisomal β-oxidation.[2,10]

The functions of CAT indicate its important roles in human health. The significance of CAT in human health was demonstrated by the discoveries that deficiency in CAT activity was present in the brain and other

tissues of a patient suffering from fatal ataxic encephalopathy[58] and in the brain, heart, and kidney of a baby with poor respiration, failure to thrive and low levels of esterified carnitine in urine.[59] CAT activity is decreased in the brains and microvessels of patients with Alzheimer's disease.[60]

A large literature exists about CAT. The advances in the area of CAT up to 1987, including physical properties, kinetics, and inhibition of CAT, have been reviewed by Colucci and Gandour.[16] Several generalizations have been made about the CAT-catalyzed acetyl transfer reaction between carnitine and CoA. The binding of carnitine and acetylcarnitine probably requires recognition of both carboxylate and quaternary ammonium portions of the molecule and may involve recognition of charge. Binding of carnitine occurs by an induced-fit mechanism and binding of CoA, or acetyl-CoA, occurs in a lock-and-key fashion. The kinetic mechanism fits a random-order equilibrium model. The molecular mechanism of acetyl transfer is probably addition-elimination with general base catalysis of hydroxy attack on a thioester in the forward reaction and general acid catalysis of oxygen expulsion in the reverse.

This section will summarize recent progress in the area of CAT enzymology.

I.2.1. CATs from different species

Table I.1 lists three CATs purified from different species in recent years. The molecular weights of these enzymes are similar to other CATs.[16]

The results of the study of Bloisi et al[48] indicate that human liver CAT is a monomer, similar to mouse liver CAT.[20] The pH optimum for activty of CATs from chick embryo liver and yeast *S. cerevisiae* are similar to most of the others, while the pH optimum for activity of CAT from human liver is higher than others.[16] Under pH 6.0, the enzyme from *S. cerevisiae* undergoes an irreversible inactivation. The substrate specificity of the enzyme from chick embryo liver is similar to that of CAT in pigeon breast muscle with maximum activity with acetyl group, but different from most of the others that utilize C_3 or C_4 acyl group as the optimal substrate.[16] The CAT from human liver has the highest activities with C_3 acyl groups, which is similar to most of the other CATs.[16]

Table I.1. CATs purified from different species

Enzyme source	Molecular weight	pH optimum	Isoelectric point	Ref.
Human liver	60,500	8.7	6.3	48
Chick embryo liver	64,000	8.0		30
Yeast *S. cerevisiae*	65,000	7.5-8.0		43

Table I.2 lists the apparent K_m values of CATs for different chain-lengths of substrates in the forward and reverse reactions. The K_m values indicate that these enzymes bind CoA (or acetyl-CoA) more tightly than they bind carnitine (or acetylcarnitine). For the CATs from chick embryo liver and human liver, the K_m values for carnitine also indicate that short-chain

acyl-CoAs are preferential substrates when the concentration of carnitine is low.

Table I.2. Values of K_m of CATs for different substrates

Enzyme source	Substrate	K_m (μM)				Ref.
		acyl-CoA	carnitine	CoA	acylcarnitine	
Human liver	C_2	21.3	97		420	48
	C_3	28.0	86		650	
	C_4	53.5	152			
	C_6	54.9	120			
	C_8	50.3	148		1390	
	C_{10}	64.0	580			
Chick embryo liver	C_2	52	160	86	800	30
	C_4	130	250	53	890	
	C_6	66	1100	78	900	
	C_8	94	2000	66	670	
S. cerevisiae	C_2	17.7	170			43

I.2.2. Amino acid sequences of CATs

A cDNA encoding for inner mitochondrial CAT of yeast *S. cerevisiae* was isolated by screening a yeast cDNA λgt11 library with antibody.[61] The whole coding sequence, which consists of 670 amino acid residues with a molecular weight of 77.3 kDa, was obtained from the cDNA and from a YEP 13 DNA clone identified using the cDNA as probe. The identity of this isolated cDNA was confirmed by using it to disrupt the yeast chromosomal CAT gene. The elimination of CAT activity from the mitochondria of the

transformed cells was shown by measuring CAT activity and by immunoblot. The acetylcarnitine content of these cells decreased significantly, indicating that no other major CAT activity remained in the cells. The residual low CAT activity in the cytosol of mutant cells did not change, suggesting that this cDNA codes for the mitochondrial isoenzyme of CAT.

The gene *YAT1*[62] from *S. cerevisiae* encodes a protein of 688 amino acids with a calculated molecular mass of 77.9 kDa, which displays significant sequence similarity to vertebrate carnitine acyltransferases and yeast inner mitochondrial CAT. The researchers of this study failed to prove, by mutant phenotype analysis, the exact substrate specificity of the encoded enzyme. Based on other evidences and data, they suggest that this novel gene is likely to code for a minor mitochondrial outer CAT.

I.2.3. Inhibition of CATs

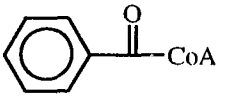
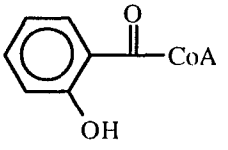
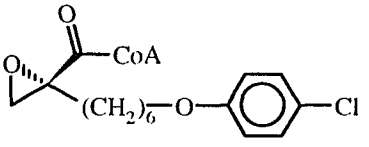
For a detailed review of inhibition of CAT up to 1987, see ref. 16.

I.2.3.1. CoA analogues

Table I.3 lists three CoA analogues that inhibit CATs from different sources.

Vessey et al[63] have studied the effect of carboxylic acid xenobiotics and their metabolites on the activity of carnitine acyltransferases and found that benzoyl-CoA and salicylyl-CoA are potent inhibitors of CAT. For the forward reaction, both benzoyl-CoA and salicylyl-CoA are competitive inhibitors

Table I.3. CoA analogues

Name	Structure	Enzyme source	Ref.
benzoyl-CoA		Sigma, St. Louis, MO, USA	63
salicylyl-CoA		Sigma, St. Louis, MO, USA	63
etomoxiryl-CoA		purified rat liver peroxisomes purified rat heart mitochondria partially purified rat liver mitochondria pigeon breast muscle (commercial)	64 64 64 64

versus acetyl-CoA with K_i values of 22 and 7.5 μM respectively. For the reverse reaction, both are competitive inhibitors versus CoA with K_i values of 17 and 9.5 μM respectively. For comparison, they determined the K_i for end-product inhibition by CoA and obtained the value of 22 μM. Benzoyl-CoA has about the same K_i as CoA. Salicylyl-CoA has a lower K_i for the enzyme than CoA or benzoyl-CoA. The results indicate that benzoate group has little effect on binding. It is not clear why the addition of a hydroxy group should improve the interaction with the enzyme.

Etomoxiryl-CoA is another CoA analogue inhibitor that was tested with CATs from different sources.[64] The data are summarized in Table I.4. The concentrations of etomoxiryl-CoA required for 50% inhibition of the different CATs are in the low micromolar range.

Table I.4. Inhibition of CATs by etomoxiryl-CoA

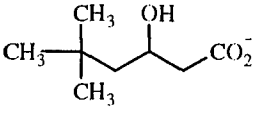
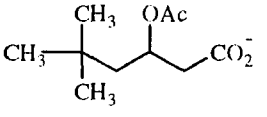
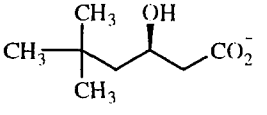
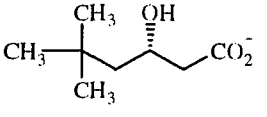
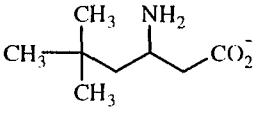
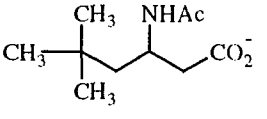
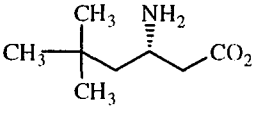
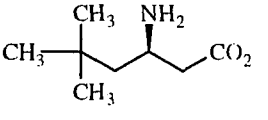
Enzyme source	K_i (μM)	Comment
Purified from rat liver peroxisomes	2	Mixed-type inhibition with acetyl-CoA
Purified from rat heart mitochondria	9	Mixed-type inhibition with acetyl-CoA
Partially purified from rat liver mitochondria	2	Mixed-type inhibition with acetyl-CoA
pigeon breast muscle (commercial)	3	Uncompetitive inhibition with acetyl-CoA

I.2.3.2. Carnitine analogues

Studies of carnitine analogues with CAT have detailed the criteria for molecular recognition at its active site.[16] Table I.5 lists several carnitine analogues that have been synthesized and assayed recently.[65,66] HDH, Ac-HDH, (*RS*)-3-amino-5,5-dimethylhexanoic acid, and (*RS*)-*N*-acetyl-3-amino-5,5-dimethylhexanoic acid are competitive inhibitors of pigeon breast CAT. (*R*)-(+)-HDH, (*S*)-(-)-HDH, (*R*)-3-amino-5,5-dimethylhexanoic acid, and (*S*)-3-amino-5,5-dimethylhexanoic acid are stereoselective competitive inhibitors of CAT. None of them is a substrate for CAT. These results indicate that the positive quaternary ammonium charge on carnitine is essential for CAT catalysis.

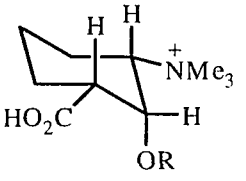
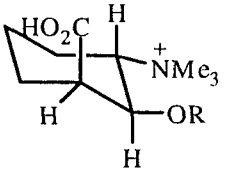
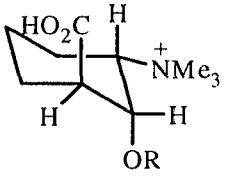
In order to determine the active conformation about C2-C3 of carnitine, Brouillette et al[67] designed cyclic carnitine analogues which contain defined spatial relationships between the quaternary ammonium, hydroxy, and carboxylate moieties (table I.6). None of them is a substrate

Table I.5. Inhibition of CAT by carnitine analogues

Name	Structure	K_i Value (μM)	Enzyme source	Ref.
(±)-3-Hydroxy-5,5-dimethyl-hexanoic acid (HDH)		8,300	Pigeon breast	65
(±)-3-Acetoxy-5,5-dimethyl-hexanoic acid (Ac-HDH)		4,100	Pigeon breast	65
(*R*)-(+)-3-Hydroxy-5,5-dimethyl-hexanoic acid (HDH)		20,300	Pigeon breast	65
(*S*)-(-)-3-Hydroxy-5,5-dimethyl-hexanoic acid (HDH)		7,500	Pigeon breast	65
(*RS*)-3-Amino-5,5-dimethyl hexanoic acid		2,600	Pigeon breast	66
(*RS*)-N-Acetyl-3-amino-5,5-dimethylhexanoic acid		24,800	Pigeon breast	66
(*S*)-3-Amino-5,5-dimethyl hexanoic acid		1,900	Pigeon breast	66
(*R*)-3-Amino-5,5-dimethyl hexanoic acid		9,200	Pigeon breast	66

for pigeon breast CAT at concentrations up to 10 mM. They are weak competitive inhibitors of CAT. The K_i values are much larger than the K_m for (*R*)-carnitine (300 μM) or (*R*)-acetylcarnitine (300 μM). The results reveal that these analogues bind much less tightly than carnitine or acetylcarnitine to CAT. The possible reasons[67] for the results are that (1) none of the conformations of the analogues represents the bound conformation for carnitine or acetylcarnitine, or (2) the extra steric bulk provided by the cyclohexyl ring residues interferes with binding.

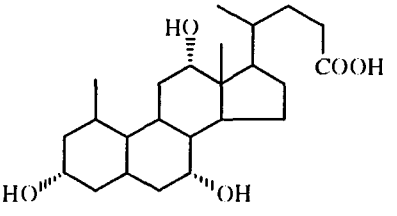
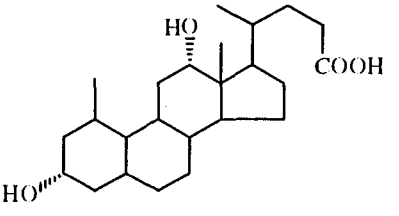
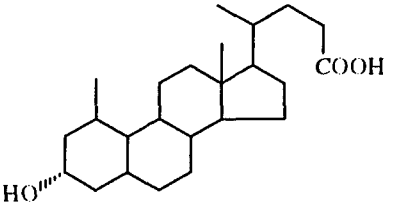
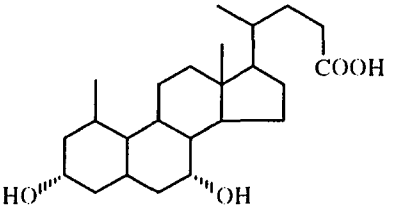
Table I.6. Inhibition of CAT by rigid carnitine analogues

Compound (racemate)	R	K_i (μM)
	H	4,100
	Ac	3,700
	H	2,900
	Ac	10,700
	H	15,900
	Ac	23,000

I.2.3.3. Bile acids

Bile acids have different inhibitory effects on CAT (Table I.7).[68,69] While determining the concentration of carnitine in rat bile, Sekas and Paul[68] have observed that the concentration increased progressively as the bile was diluted, which prompted them to study inhibition of bile acids on

Table I.7. Bile acids as inhibitors of CAT

Name	Structure	Enzyme source	Ref.
cholic acid		purified pigeon breast muscle	68
deoxycholic acid		purified pigeon breast muscle	68
lithocholic acid		purified pigeon breast muscle	68
chenodeoxycholic acid		purified pigeon breast muscle rat liver peroxosomes (*in vitro*)	68 69

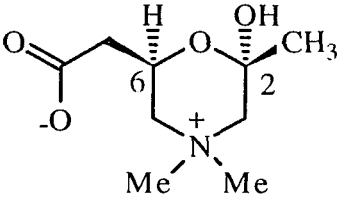
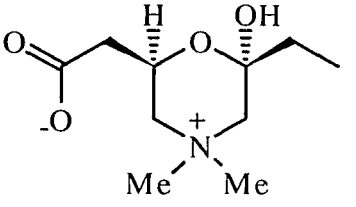
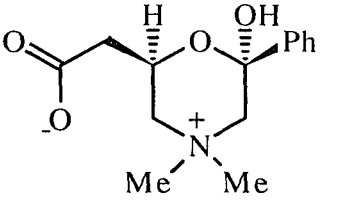
CAT. They have examined inhibitory effects of major bile acids found in bile. All bile acids tested (Table I.7) inhibit CAT to varying degrees. At physiological concentrations, the degree of inhibition is chenodeoxycholic > cholic > deoxycholic > lithocholic acid. For the forward reaction, K_i value for chenodeoxycholic acid is 520 μM. The inhibition is competitive with carnitine. The reverse reaction is inhibited by 25, 30, 63, and 89% in the presence of 0.23, 0.45, 0.90, and 1.6 mM chenodeoxycholic acid, respectively. They have also studied inhibition of CAT by bile acids in rat liver peroxisomes.[69] The apparent K_i value is 890 μM. Inhibition is observed both in vitro and ex vivo.

I.2.3.4. Conformationally constrained reaction-intermediate analogues

This type of inhibitors is the most powerful tool in probing the structure of enzyme binding sites while they are in the conformations adopted for catalysis. Several of this type of inhibitors have been synthesized and tested.[16] Table I.8 lists three analogues that have been tested recently.[70] Table I.9 lists their inhibition constants with various CATs. All these hemiacylcarnitiniums are competitive inhibitors. HAC binds better than (*R*)-carnitine, (*R*)-acetylcarnitine, or CoA to CAT. HBC weakly inhibits CATs. It binds to CATs less tightly than do the substrates. The degree of inhibition is HAC > HPrC > HBC due to the different substitution groups on C2 (methyl, ethyl, and phenyl group). Nonracemic HAC inhibits CAT more

strongly than racemic HAC,[71] indicating the importance of configurations of C2 and C6 in enzyme recognition.

Table I.8. Conformationally constrained reaction-intermediate analogues

Name	Structure
(*2S,6R*)-6-(Carboxylatomethyl)-2-hydroxy-2,4,4-trimethyl-morpholinium (hemiacetylcarnitinium, HAC)	
(*2S,6R*)-6-(Carboxylatomethyl)-2-ethyl-2-hydroxy-4,4-dimethylmorpholinium (hemipropanoylcarnitinium, HPrC)	
(*2S,6R*)-6-(Carboxylatomethyl)-2-hydroxy-4,4-dimethyl-2-phenylmorpholinium (hemibenzoylcarnitinium, HBC)	

The inhibitory activity of HAC is explained by its structural similarity to the tetrahedral-intermediate proposed in the acetyl transfer between CoA and acetylcarnitine (Figure I.1).[71,72]

Table I.9. Inhibition constants of hemiacylcarnitiniums with CAT

Enzyme source	Substrate	K_m(μM)	Inhibitor	K_i(μM)
Rat liver peroxisomes	(*R*)-Acetylcarnitine	290 ± 20	HAC	69 ± 5
	(*R*)-Carnitine	143		
Rat heart mitochondria	CoA	130 ± 20		92 ± 14
Pigeon breast	(*R*)-Carnitine	120	HPrC	200 ± 30
	(*R*)-Acetylcarnitine	350		
Pigeon breast	(*R*)-Carnitine	120	HBC	3500 ± 500
Rat heart mitochondria	CoA	120 ± 10		2400 ± 70
Rat liver peroxisomes	(*R*)-Acetylcarnitine	280 ± 10		1670 ± 70

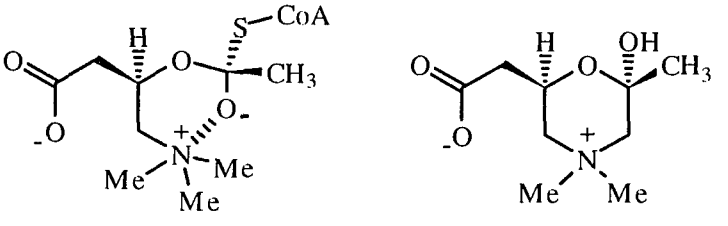
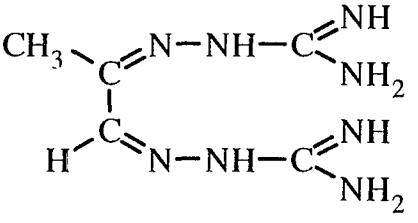


Figure I.1. Structural relationship between the proposed tetrahedral-intermediate in the acetyl transfer reaction and the structure of HAC

I.2.3.5. Other inhibitors

Methylglyoxal bis(guanylhydrazone) (MGBG) inhibits purified pigeon breast CAT with K_i value of 1.6 mM.[73] It is competitive with (*R*)-carnitine. The competitive inhibition of MGBG with carnitine is probably due to the structural similarity between MGBG and carnitine or acetylcarnitine as proposed by Brady et al.[73] The guanidium group occupies the quaternary

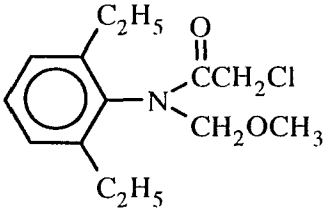
ammonium site and the chain extends in a similar fashion to carnitine with an imine group occupying a position similar to the acyl carbonyl. In this location, MGBG can form a thioaminal with either CoA or cysteine present in the active site.



MGBG

Human liver CAT[48] and chick embryo liver CAT[30] are inhibited by Ca^{2+} which doesn't inhibit other avian and mammalian CATs. [16]

Mouse liver CAT is inhibited by alachlor, a herbicide.[74]



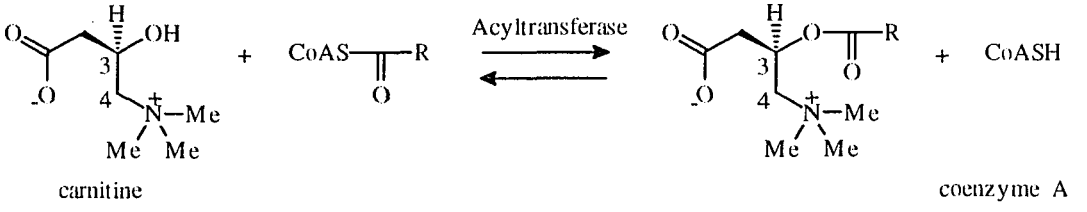
alachlor

I.2.4. Summary

Some ideas are further confirmed. CAT enzymatic catalysis requires a positive quaternary ammonium charge on carnitine. The chiralities of (acetyl)carnitine and the proposed tetrahedral-intermediate are important for CAT catalysis.

I.3. DESIGN OF INHIBITORS OF CARNITINE ACYLTRANSFERASE

The carnitine acyltransferases are a family of enzymes that differ with respect to subcellular location, substrate specificity, and sensitivity to inhibitors.[2] They catalyze acyl transfer between carnitine and CoA (Scheme I.2). The three-dimensional structures of these proteins are unknown.



Scheme I.2

In order to probe the structures of the molecular interactions between carnitine and the active sites in these enzymes, we have designed conformationally constrained analogues to mimic the proposed reaction-intermediate for acyl transfer between carnitine and CoA. By comparing inhibition constants (K_i's) of a series of conformationally constrained analogues, we can identify the topographical arrangement of the key recognition sites on these enzymes.

I.3.1. Considerations in the enzyme inhibitor design

Several considerations must be made when designing conformationally constrained reaction-intermediate analogue inhibitors.

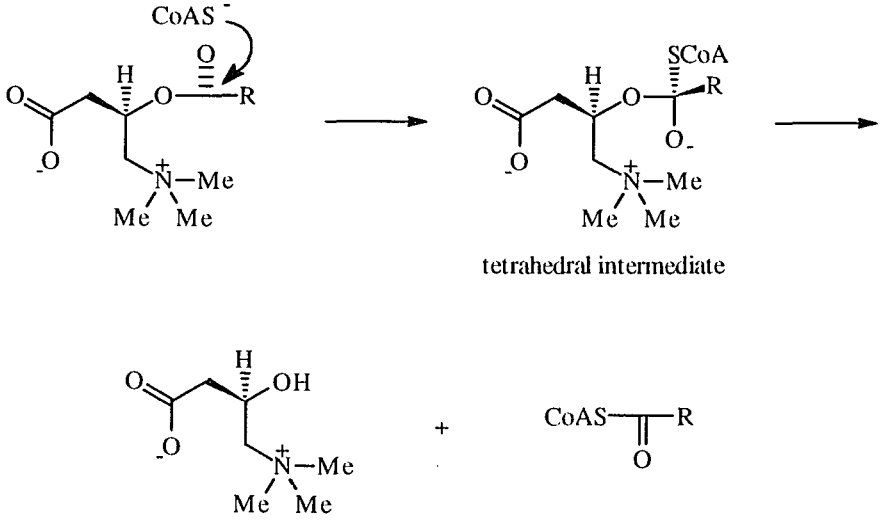
First, we must consider the possible enzyme recognition sites in the substrates. The enzymes, especially CAT,[75] recognize trimethylammonium, carboxylate, and acyl group on acylcarnitine. Recent studies[65,66] suggest that the positive quaternary ammonium charge on carnitine is essential for CAT catalysis. The enzymes also recognize CoA.

Second, the conformations of the substrates must be considered. There are nine possible conformations for carnitine. To design a conformationally constrained analogue, we need to know which of the nine conformations of carnitine is bound to the enzyme. The conformational analyses of carnitine and acetylcarnitine have been performed by using single crystal X-ray diffraction,[72] NMR, and molecular mechanics (MM2).[76] Those structural studies[72,76] show that carnitine and acetylcarnitine strongly prefer gauche (-) conformation about N-C4-C3-O torsion angle.

Based on these conformational analyses of carnitine and acetylcarnitine, we can lock the N-C4-C3-O torsion angle in the gauche (-) conformation by formation of a ring and probably not lose much in binding to the enzyme. These analogues must have groups necessary for enzyme recognition anchored to the conformationally constrained molecular framework in a well-defined stereochemistry.

Third, the enzyme inhibitors should mimic the structure of a reaction-intermediate or a transition state of the mechanism. Wolfenden[77] has pioneered the development of transition-state analogue

inhibitors of enzymes. The idea is that enzymes bind transition structures or reaction-intermediates more tightly than reactants or products. Molecules that resemble the structures of transition states or reaction-intermediates, but are unreactive, will bind strongly to the enzymes. A mechanism for acetyl transfer in CAT has been proposed[72] (Scheme I.3) based on chemical model studies of O-to-S acyl transfer.[78] It is presumed that a similar mechanism operates in COT and CPT.[79]



Scheme I.3

In forming such a tetrahedral-intermediate, the thiolate should approach the acyloxy from the less-congested side (carboxylate on carnitine "folded" back). This attack arrow is on the *Re* face of the ester, presuming that the acyloxyl group is in the most stable conformation.[76] This *R* configuration of the tetrahedral-intermediate can adopt a

conformation that favors intramolecular electrostatic catalysis.[80] The developing negative charge on the carbonyl oxygen would be stabilized by the positively charged trimethylammonium group. The enzymes also recognize the configuration at C3 of (acyl)carnitine during the acyl transfer. A reaction-intermediate analogue must mimic both of these configurations.

I.3.2. Design of reaction-intermediate analogues

Conformationally constrained analogues are the most effective for examining the topography of an active site in an enzyme when catalysis occurs. Based on the studies on the possible binding sites in (acyl)carnitine, the conformational studies on carnitine and acetylcarnitine, and the analysis of the possible mechanism for acyl transfer, we have designed the reaction-intermediate analogues as shown in Figure I.2. The morpholinium ring adopts a chair conformation. Therefore, the N-C5-C6-O torsion angle in the morpholinium ring is

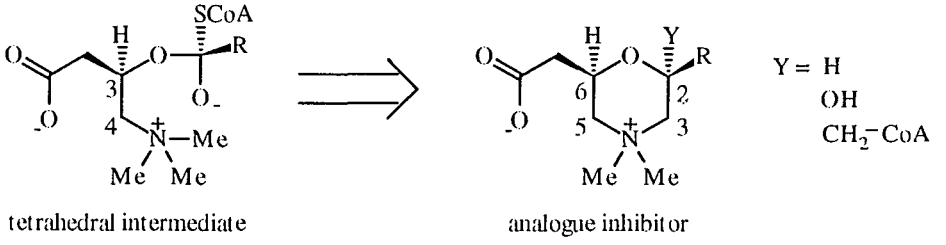


Figure I.2. Proposed tetrahedral-intermediate and analogue inhibitor

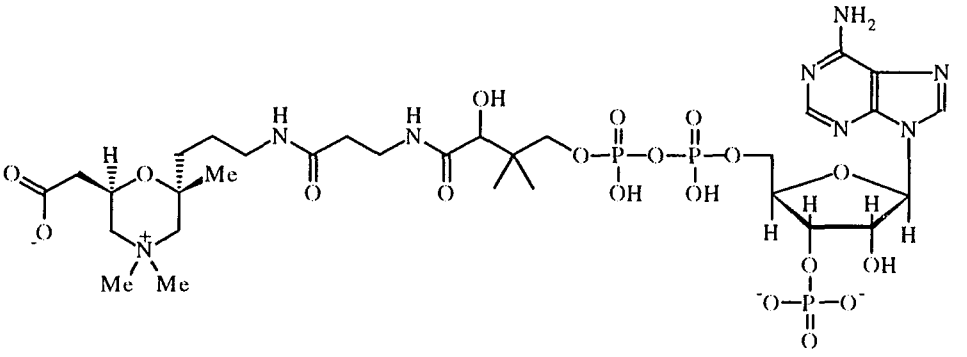
locked in a gauche (-) conformation, which is similar to N-C4-C3-O torsion angle in (acyl)carnitine. The carboxymethyl and the alkyl chain (R) are *cis* diequatorial in the most stable conformation. A covalent bond (C2-C3) in the morpholinium ring replaces the electrostatic interaction between the negatively charged carbonyl oxygen and the positively charged trimethylammonium group in the reaction-intermediate. A six-membered ring was chosen because of ease of synthesis and fewer conformations.

Several conformationally constrained cyclic analogues, made in this laboratory, exhibit inhibitory activities toward different carnitine acyltransferases.[16,70,79,81] For example, (+)-hemiacetylcarnitinium (HAC, R = CH_3, Y = OH, Figure I.2) inhibits rat liver peroxisomal CAT and rat heart mitochondrial CAT;[70] (+)-hemipalmitoylcarnitinium (HPC, R = $(CH_2)_{14}CH_3$, Y = OH) inhibits mitochondrial CPT-II and peroxisomal COT of rat liver,[79] and rat heart and rat liver mitochondrial CPT-I.[81]

In these analogues, the location of Y (OH) is where CoA attaches to the proposed reaction-intermediate. In order to test this idea and increase the potency of the inhibitors, we need a plan to synthesize analogues where Y = CH_2-CoA.

I.4. PROJECT GOAL

The ultimate goal of this project is to synthesize an analogue, **I**, of the proposed tetrahedral-reaction-intermediate for acetyl transfer between carnitine and CoA. This analogue contains both carnitine and

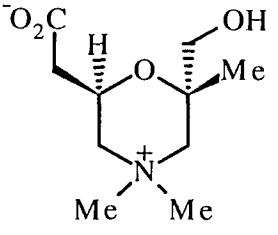


I

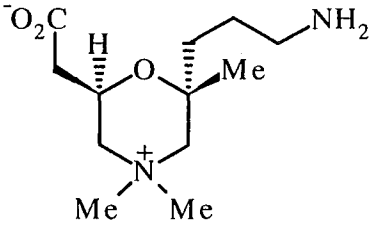
CoA fragments (Y = CH_2-CoA); those previously synthesized contain only the carnitine fragment and hydroxy group (Y = OH). The morpholinium ring in this analogue contains two chiral centers, so there are four stereoisomers. The previously synthesized analogue inhibitors are either racemic mixture or only one stereoisomer. The lability of the hemiketal linkage allows the carbon to equilibrate to the more stable configuration.[70] In these molecules, hydroxy group prefers to be axial. The goal of this project is to synthesize all the four stereoisomers of the reaction-intermediate analogue. By comparing the inhibitory effects of these four stereoisomers, more information about the topographical arrangement of the key recognition sites on the enzymes can be obtained. This information will lead to a three-dimensional picture of the active sites of the enzymes.

I.5. SPECIFIC GOAL

The goal of my research is to develop synthetic strategies for four stereoisomers of 6-carboxylatomethyl-2-hydroymethyl-2, 4, 4-trimethyl morpholinium, **II**, which are the key synthetic intermediates in the proposed route to the four stereoisomers of 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethylmorpholinium, **III**, which will serve as precursors to **I**. The assay of those synthesized enzyme inhibitors by Drs. Nóirín Nic a' Bháird and Rona R. Ramsay at UCSF is also a part of this work.



II



III

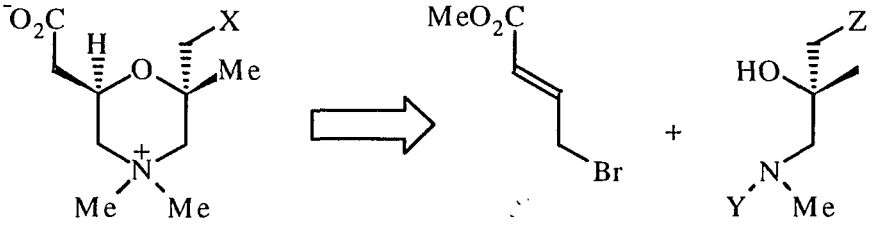
CHAPTER II

SYNTHESES OF INHIBITORS OF CARNITINE ACETYLTRANSFERASE

II.1. RETROSYNTHETIC ANALYSIS

The syntheses of the target molecules can be approached from different pathways. Herein, we examine the structures of all the target molecules with different retrosynthetic strategies to analyze the possible pathways to approach these molecules.

Both the target molecules **II** and **III** were disconnected into two main components: methyl 4-bromo-2-butenoate and β-hydroxyamine, which carries different functional groups for **II** and **III** and can be made in different ways (Scheme II.1).

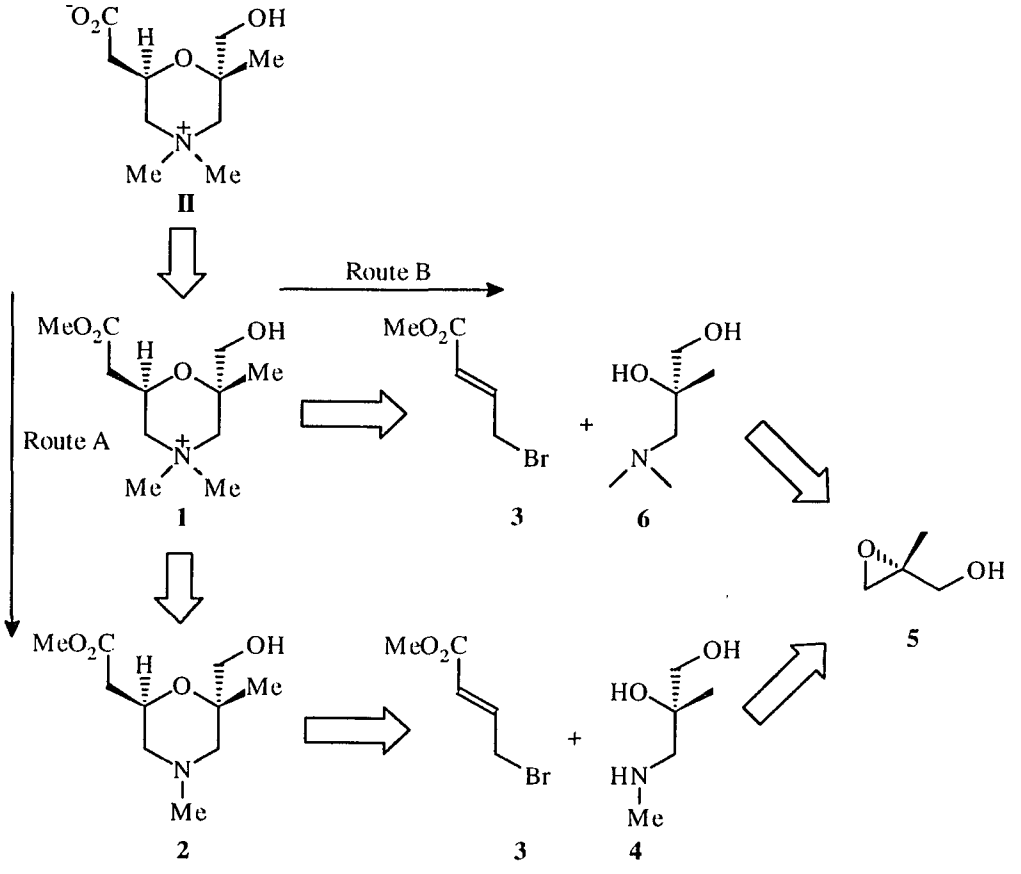


X = OH, $CH_2CH_2NH_2$ Y = H, Me Z = OH, CH_2CN

Scheme II.1

II.1.1. Target molecule II

Two retrosynthetic routes were proposed for synthesizing target molecule **II**, 6-carboxylatomethyl-2-hydroxymethyl-2, 4, 4-trimethyl morpholinium, as shown in Scheme II.2.



Scheme II.2

In retrosynthetic route A, methylation of the tertiary amine, methyl 2-(6-hydroxymethyl-4,6-dimethylmorpholinyl)acetate, **2**, followed by hydrolysis of methyl ester would give the target molecule **II**. Compound **2** is disconnected into two parts: methyl 4-bromo-2-butenoate, **3**, and 3-

methylamino-2-methylpropane-1,2-diol, **4**. Compound **3** is commercially available. Compound **4** can be made by ring opening of 2-methylglycidol, **5**, with methylamine. Compound **5** is commercially available for both *R* and *S* configurations. Ring closure of **3** with (*R*)-**4** or (*S*)-**4** would give a diastereomeric mixture of **2**, which could be separated by chromatography.

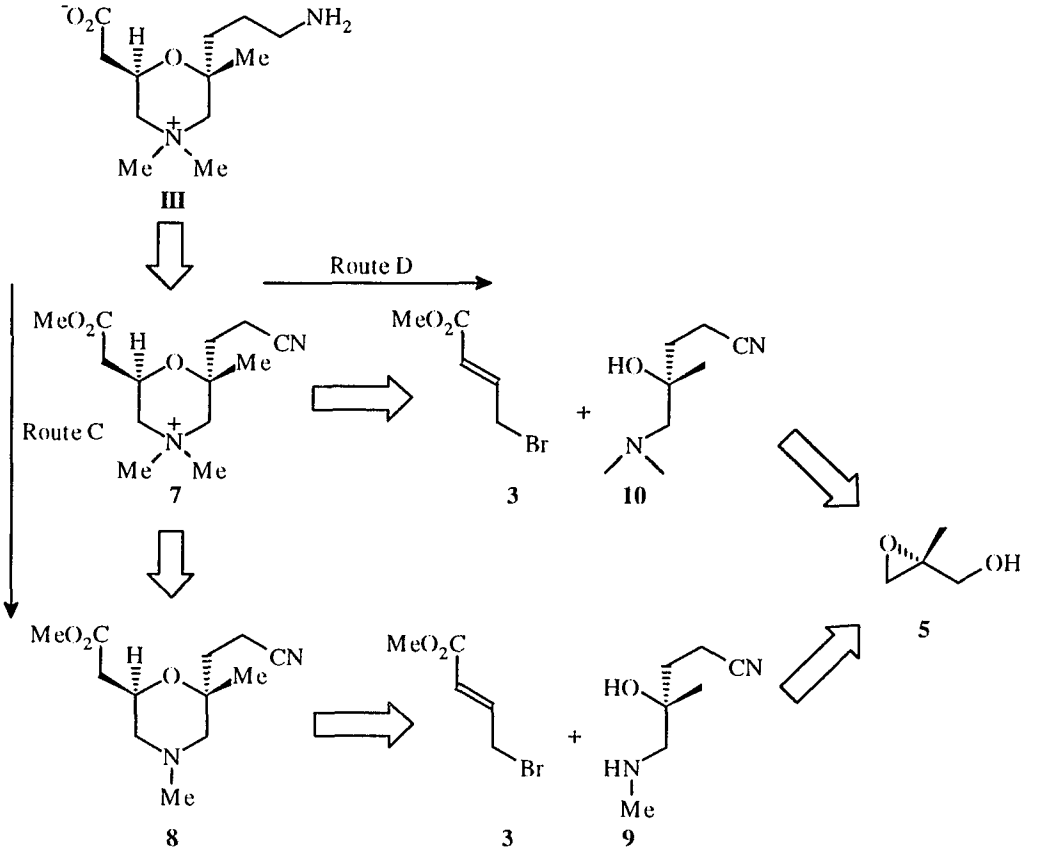
In retrosynthetic route B, 2-hydroxymethyl-6-(methoxycarbonyl)-methyl-2,4,4-trimethylmorpholinium, **1**, is disconnected into **3** and 3-dimethylamino-2-methylpropane-1,2-diol, **6**, which could be made from (*R*)-**5** and (*S*)-**5** by ring opening with dimethylamine. In this route, methylation of tertiary amine is not needed. So it seems shorter than route A. But ring closure of **3** with (*R*)-**6** or (*S*)-**6** would give a diastereomeric mixture of quaternary amine salts, **1**, which might be much more difficult to separate than tertiary amine, **2**.

II.1.2. Target molecule III

Schemes II.3, II.4, and II.5 show the retrosynthetic routes for synthesizing target molecule **III**, 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethylmorpholinium.

In retrosynthetic route C (Scheme II.3), methylation of tertiary amine of methyl 2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**, followed by hydrolysis of methyl ester and reduction of cyano group would give the target molecule **III**. Compound **8** is disconnected into **3** and 5-methylamino-4-hydroxy-4-methylpentanenitrile, **9**, which could be made by conversion of

the hydroxy group of **5** into a cyanomethyl group and ring opening of the epoxy with methylamine.

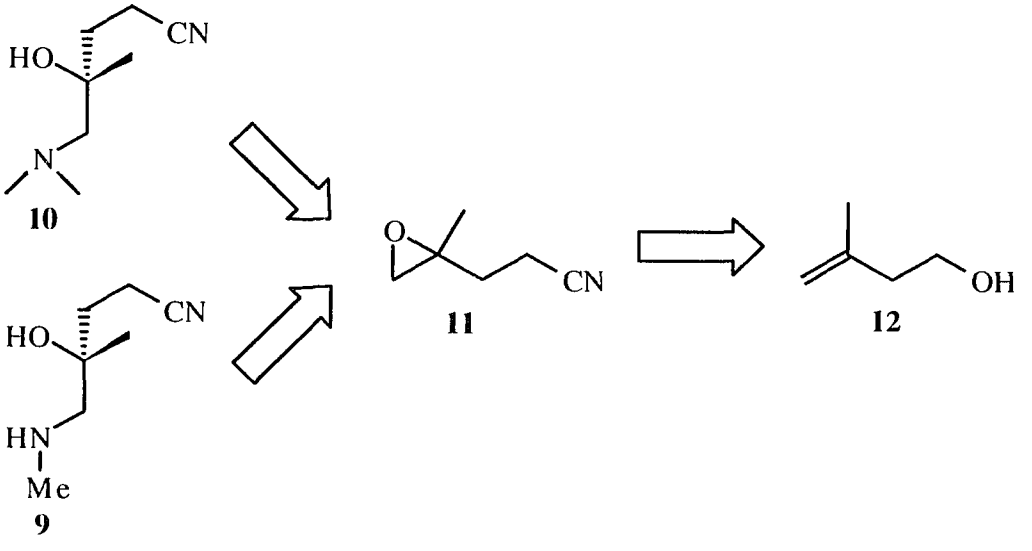


Scheme II.3

In retrosynthetic route D (Scheme II.3), 2-(2-cyanoethyl)-6-(methoxycarbonyl)methyl-2,4,4-trimethylmorpholinium, **7**, is disconnected into **3** and 5-dimethylamino-4-hydroxy-4-methylpentanenitrile, **10**, which could be made the same way as compound **9** using dimethylamine in place of methylamine. Ring closure of **3** with **10** would give a diastereomeric mixture

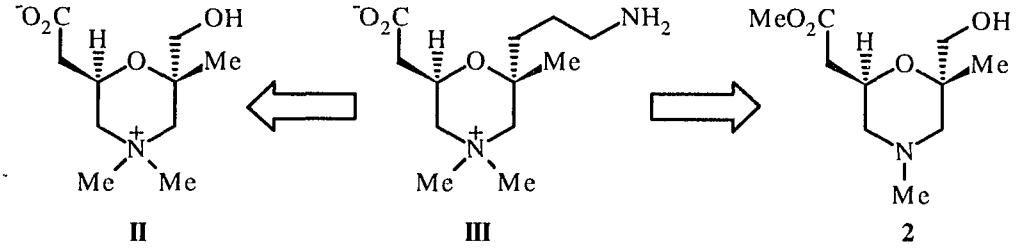
of quaternary amine salts **7**, which might be much more difficult to separate than compound **8**.

Scheme II.4 shows other retrosynthetic routes for making **10** and **9**. Compounds **10** and **9** could be made by ring opening of 3-(1-methyloxiranyl)propanenitrile, **11**, with dimethylamine and methylamine respectively. Compound **11** could be derived from 3-methyl-3-buten-1-ol, **12**, which is commercially available. In these two routes, we have to resolve **11** or **10** and **9**.



Scheme II.4

In Scheme II.5, the target molecule **III** could be derived from **II** or **2**, which could be made according to Scheme II.2.



Scheme II.5

II.1.3. Summary

For target molecule **II**, both routes A and B originate from the same starting materials, (*R*)- and (*S*)-2-methylglycidol, **5**, which are commercially available. Chiral compounds (*R*)- and (*S*)-3-methylamino-2-methylpropane-1,2-diol, **4**, and (*R*)- and (*S*)-3-dimethylamino-2-methylpropane-1,2-diol, **6**, could be derived from these two chiral starting materials. The ring closure products of methyl 4-bromo-2-butenoate, **3**, with diols **4** or **6** would be mixtures of diastereomers **2** and **1**, respectively. The diastereomeric mixture of quaternary amine salts, **1**, would be much more difficult to separate than the diastereomeric mixture of tertiary amines, **2**. Therefore, route A would be better than route B.

For target molecule **III**, both routes C and D originate from the same starting materials. In route D, we would have the same problem as that in Scheme II.2, route B for target molecule **II** — the separation of diastereomeric mixture of quaternary salts. In the routes shown in Scheme II.4, we need to resolve 3-(1-methyloxiranyl)propanenitrile, **11**, 5-

methylamino-4-hydroxy-4-methylpentanenitrile, **9**, or 5-dimethylamino-4-hydroxy-4-methylpentanenitrile, **10**. If route A in Scheme II.2 for target molecule **II** works well, the routes in Scheme II.5 for target molecule **III** might work.

By routes C or D, we can build up the side chain (cyanomethyl) before the ring-closure step, which would have lower yield than the other steps because of potential side reactions and chromatography to separate diastereomers. Therefore, we chose to work on routes C and D before A and B. Compound **5** is not optically pure and needs optical enrichment. But after the optical enrichment by converting it into 4-nitrobenzoate ester followed by recrystallization, it is very difficult to get it back. We have tried the route in Scheme II.4. Racemic **10** can be made from **12** and can be resolved as the 3,5-dinitrobenzoate esters by HPLC using a chiral column. For preparative scale, this resolution method is tedious and time consuming. Therefore, we have switched to routes A and B.

II.2. RESULTS AND DISCUSSION

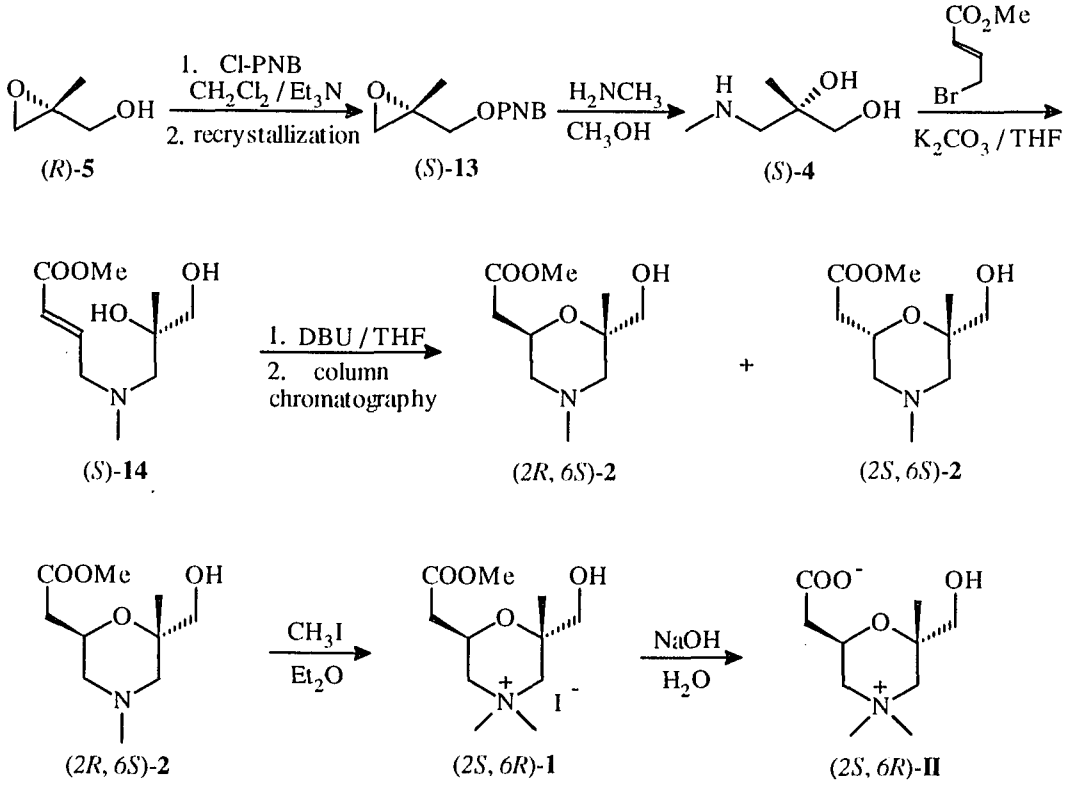
Our synthetic efforts can be divided into two parts:

1) syntheses of (*2R,6R*)-, (*2S,6S*)-, (*2R,6S*)-, and (*2S,6R*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, **II**.

2) syntheses of methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**.

II.2.1. Syntheses of (*2R,6R*)-, (*2S,6S*)-, (*2R,6S*)-, and (*2S,6R*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, II

The syntheses of four stereoisomers of 6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, target molecule **II**, are illustrated in Scheme II.6.



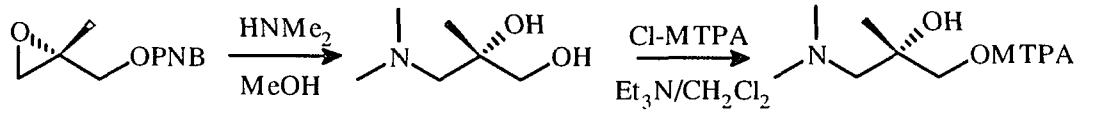
Scheme II.6

According to the retrosynthetic analysis in Scheme II.2, (*R*)- and (*S*)-3-methylamino-2-methylpropane-1,2-diol, **4**, could be made from (*S*)- and (*R*)-2-methylglycidol, **5**, respectively by ring opening with methylamine; (*R*)- and

(*S*)-3-dimethylamino-2-methylpropane-1,2-diol, **6**, could be made from (*S*)- and (*R*)-2-methylglycidol, **5**, respectively by ring opening with dimethylamine. Compound **5** is commercially available for both *R* and *S* configurations.

Before starting our synthesis, we measured the optical purities of (*R*)- and (*S*)-**5** by ^{1}H NMR analysis of their Mosher's esters.[82] We found that their optical purities were only about 90%. We needed the optical purities of our compounds to be greater than 97%. Therefore, the first step in the synthesis was optical enrichment of **5**. Gao et al[83] showed that the optical purity of **5** can be improved by recrytallizing the 4-nitrobenzoate ester. As shown in Scheme II.6, compound **5** was readily converted into (1-methyloxiranyl)methyl 4-nitrobenzoate, **13**, by treatment with 1 equivalent of 4-nitrobenzoyl chloride in dichloromethane in the presence of 1.1 equivalents of triethylamine at 0 °C. Successive recrystallizations from ethanol, diethyl ether, and isopropyl ether gave optically enriched ester **13** in 49% yield.

The optical purity of **13** was determined by ^{1}H NMR analysis (Scheme II.7). Compound **13** was converted into **6** by reaction with 4 equivalents of

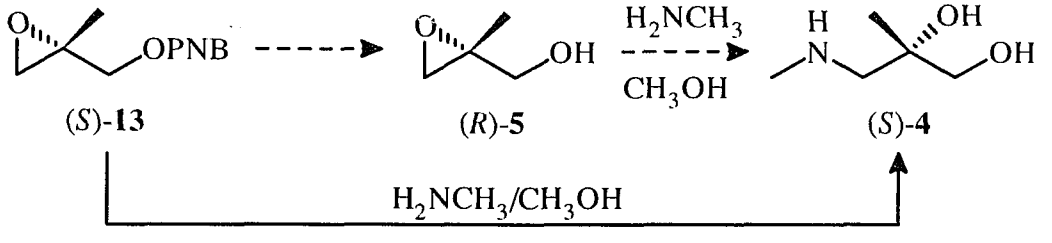


13 **6** **15**

Scheme II.7

dimethylamine in methanol. In this step, two reactions occur, ring opening of epoxide and cleavage of 4-nitrobenzoate, giving **6**. The product of the 4-nitrobenzoate ester cleavage was methyl 4-nitrobenzoate instead of *N,N*-dimethyl 4-nitrobenzoate amide. This ester was removed by concentration of the reaction mixture followed by filtration. Then compound **6** was converted into Mosher's ester **15** by treatment with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl). ^{1}H NMR analysis of **15** indicated the optical purities of both (*R*)- and (*S*)-**13** greater than 97% (the minor isomer was undetectable). Compound **6** was characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analysis.

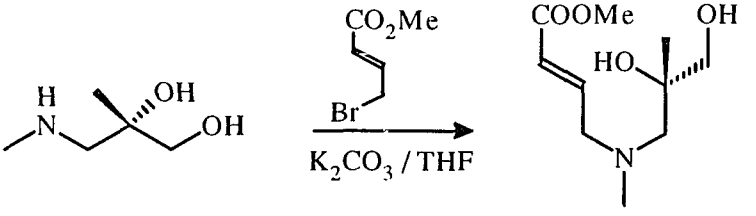
After the optical enrichment, compound **5** could be recovered by hydrolysis of **13**. Then ring opening of **5** with methylamine would give **4** (Scheme II.8). So, two steps would be needed to convert **13** into **4**. We thought that the ester bond of **13** could be cleaved by aminolysis with methylamine, just as it was from **13** to **6** with dimethylamine, so that the cleavage of the ester bond and ring opening of epoxy might take place in one step. This way, we could save one step.



Scheme II.8

Based on this idea, we tried the reaction of **13** with methylamine (Scheme II.8). A solution of **13** in methanol was added into a solution of 6 equivalents of methylamine in methanol at room temperature and the mixture was stirred for 3 hours. *N*-Methyl 4-nitrobenzoate amide produced from the reaction was insoluble in methanol and was removed by concentration of the reaction mixture followed by filtration. Compounds (*R*)- and (*S*)-**4** were obtained from (*R*)- and (*S*)-**13** respectively in 75% yield and characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analyses.

(*R*)- and (*S*)-Methyl 4-[methyl-(2,3-dihydroxy-2-methylpropyl)amino]-2-butenoate, **14**, were prepared by reaction of (*R*)- and (*S*)-**4** with 1 equivalent of methyl 4-bromo-2-butenoate in the presence of potassium carbonate in THF at room temperature overnight (Scheme II.9). At least 1 equivalent of potassium carbonate was needed to neutralize hydrogen bromide produced from the reaction. After completion of the reaction, the precipitate was removed by filtration. Concentration of the solution gave crude product **14** in 83% yield, which was used for next reaction without

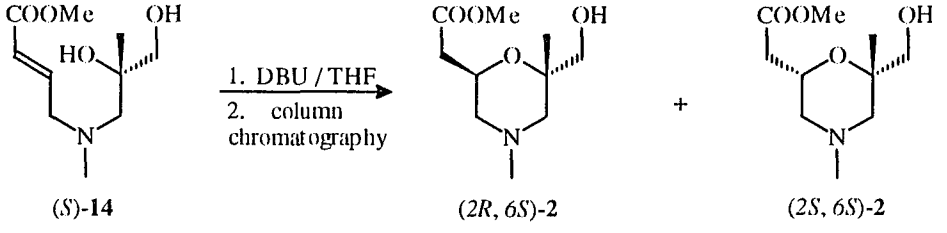


(*S*)-**4** (*S*)-**14**

Scheme II.9

further purification. From ^{1}H NMR spectra of the crude product, we could see that the reaction was a little cleaner in THF than in diethyl ether. Compound **14** was characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and mass spectroscopy.

Ring closure of **14** in THF was catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[84] to give methyl 2-(6-hydroxymethyl-4,6-dimethylmorpholinyl)acetate, **2**, as a diastereomeric mixture which was separated by column chromatography (silica gel, 230-400 mesh, hexanes:dichloromethane:ethanol = 100:100:30) to give two diastereomers with a ratio of 3:1 and total isolated yield of 40% (Scheme II.10). In this reaction, 6-membered ring was formed instead of 7-membered ring because 6-membered ring is more stable and easily formed than 7-membered ring.[85] The major isomer was the one with the (methoxycarbonyl)methyl and hydroxymethyl adopting *trans* positions. From (*S*)-**14**, we got (*2R,6S*)- and (*2S,6S*)-**2**. From (*R*)-**14**, we got (*2S,6R*)- and (*2R,6R*)-**2**. The four stereoisomers of **2** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analyses.



(*S*)-**14** (*2R, 6S*)-**2** (*2S, 6S*)-**2**

Scheme II.10

The resulting stereoselectivity may be attributed to chair transition structures in the ring closure reaction (Figure II.1). The transition structure on the right is more stable and easily formed than that on the left due to the intramolecular interaction between the hydrogen of hydroxy group and the nitrogen. Therefore, the transition structure on the right is favored and the major isomer of the product is the one with the (methoxycarbonyl)methyl and hydroxymethyl adopting *trans* positions.

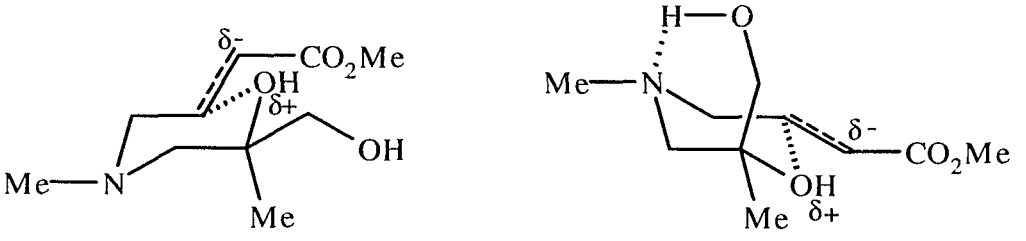


Figure II.1. Transition structures for ring closure reaction of **14**

Treatment of **2** with excess of iodomethane in diethyl ether at room temperature gave 2-hydroxymethyl-6-(methoxycarbonyl)methyl-2,4,4-trimethylmorpholinium iodide, **1**, as a light yellow solid in 74% yield. At least 15 equivalents of iodomethane were needed for the completion of the reaction. Crystals for X-ray analysis were obtained by recrystallization from methanol by vapor diffusion with diethyl ether. The four stereoisomers of **1** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analyses, and verified by single-crystal X-ray analyses (Figure II.2 and II.3).

In crystals, the morpholine ring of **1** is in a chair conformation. For (*2R,6S*)-**1** and its enantiomer (*2S,6R*)-**1**, the (methoxycarbonyl)methyl and methyl on C3* are *cis*; both occupy equatorial positions. For (*2S,6S*)-**1** and its enantiomer (*2R,6R*)-**1**, the methoxycarbonylmethyl and hydroxymethyl are *cis*; both occupy equatorial positions. The (methoxycarbonyl)methyl always occupies an equatorial position for all four stereoisomers.

Treatment of **1** with 1 equivalent of sodium hydroxide solution (0.1 N) gave the target molecule **II**, 6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, which was recrystallized from methanol/acetone (1/11) as colorless crystals in 67% yield. The four stereoisomers of **II** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analyses, and verified by single-crystal X-ray analyses (Figure II.4 and II.5).

In crystals, the morpholine ring of **II** adopts a chair conformation. For (*2R,6S*)-**II** and its enantiomer (*2S,6R*)-**II**, the carboxylatomethyl and methyl on C3 are *cis*; both occupy equatorial positions. For (*2S,6S*)-**II** and its enantiomer (*2R,6R*)-**II**, the carboxylatomethyl and hydroxymethyl are *cis*; both occupy equatorial positions. The carboxylatomethyl always occupies an equatorial position for all four stereoisomers.

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* The numbering system for the ORTEP drawings of the crystal structures is different from IUPAC nomenclature.

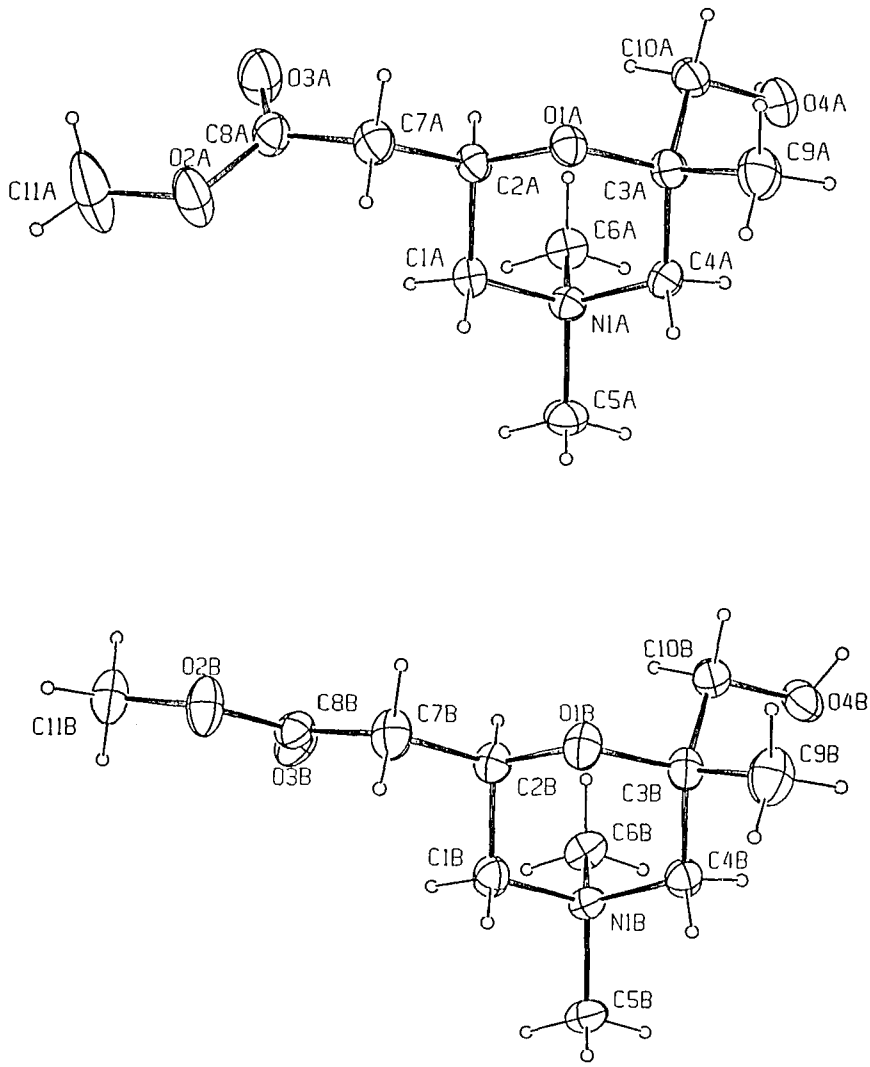


Figure II.2. ORTEP drawing of (2*S*,6*R*)-2-hydroxymethyl-6-methoxycarbonylmethyl-2,4,4-trimethylmorpholinium iodide

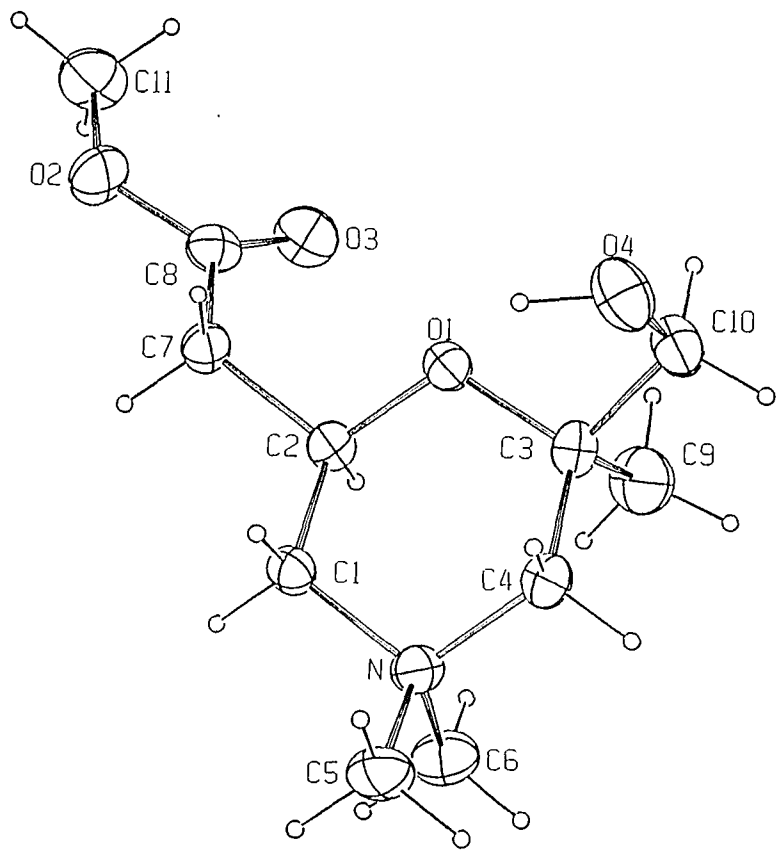


Figure II.3. ORTEP drawing of (*2R,6R*)-2-hydroxymethyl-6-methoxycarbonylmethyl-2,4,4-trimethylmorpholinium iodide

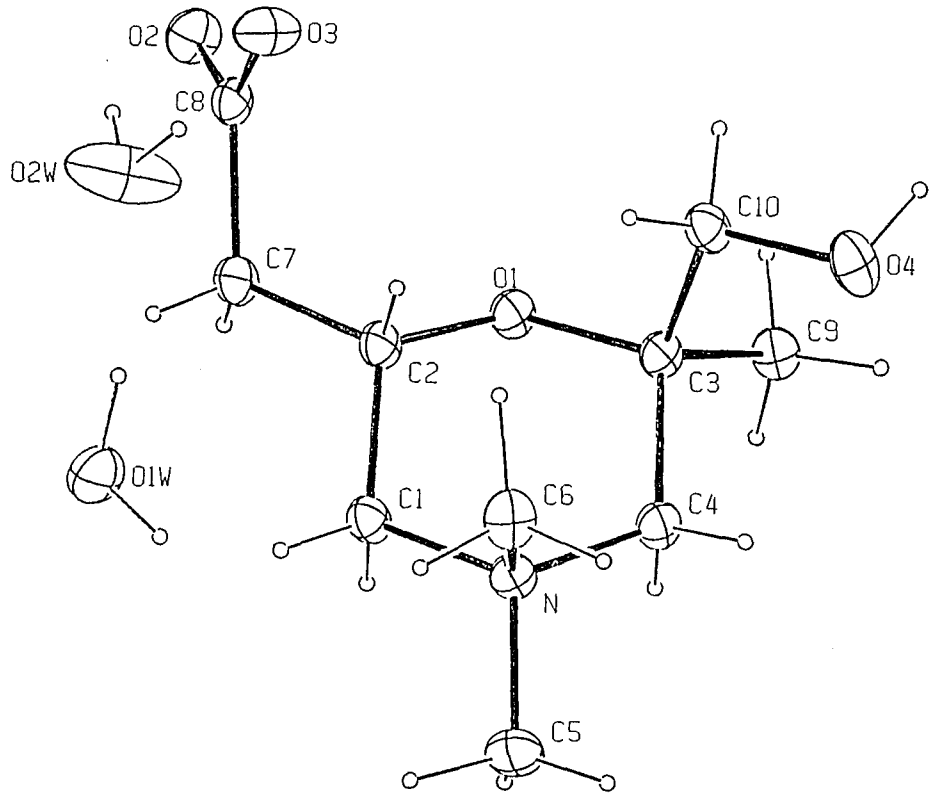


Figure II.4. ORTEP drawing of (*2R,6S*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium

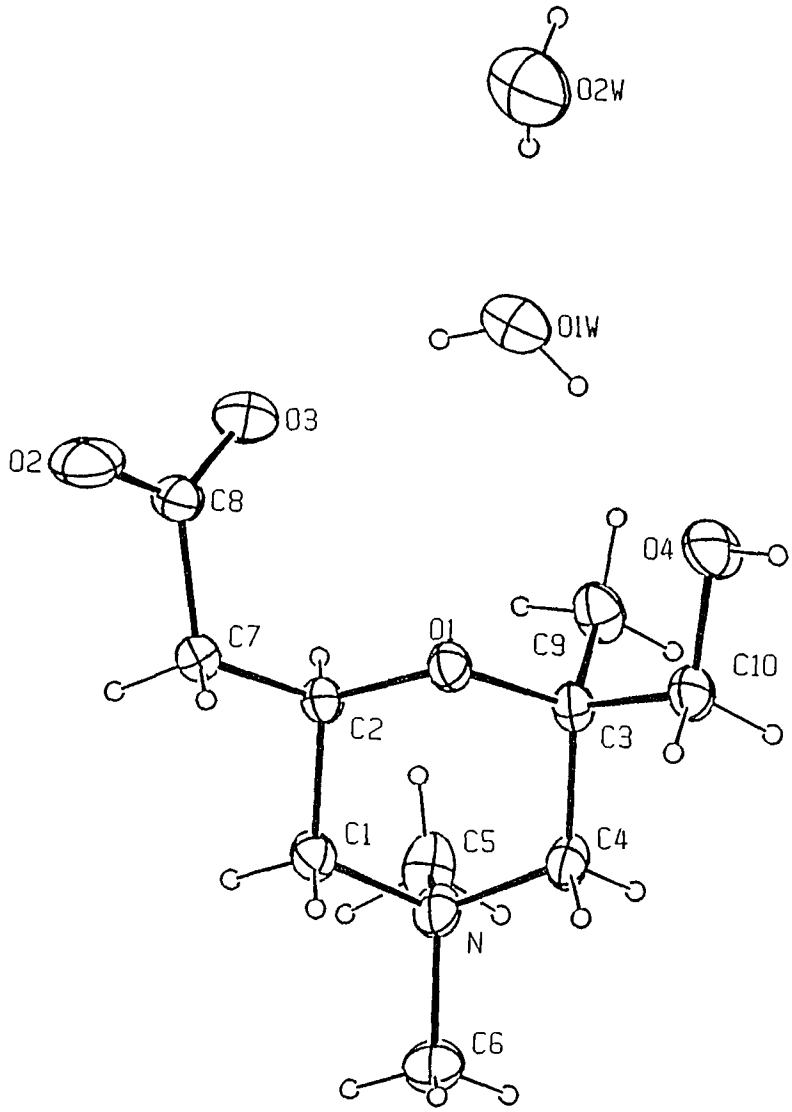
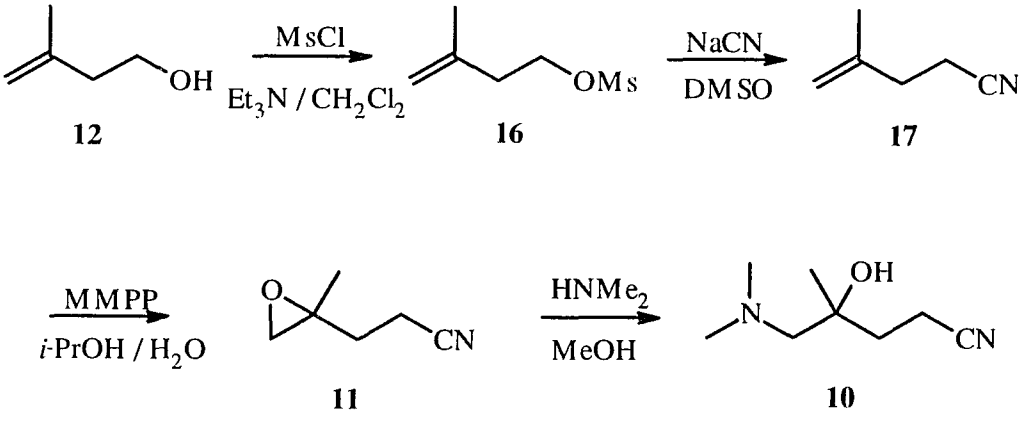


Figure II.5. ORTEP drawing of (*2R,6R*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium

II.2.2. Syntheses of methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, 8

II.2.2.1. Preparation of (*RS*)-5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, (*RS*)-10

Preparation of (*RS*)-5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, is shown in Scheme II.11.



Scheme II.11

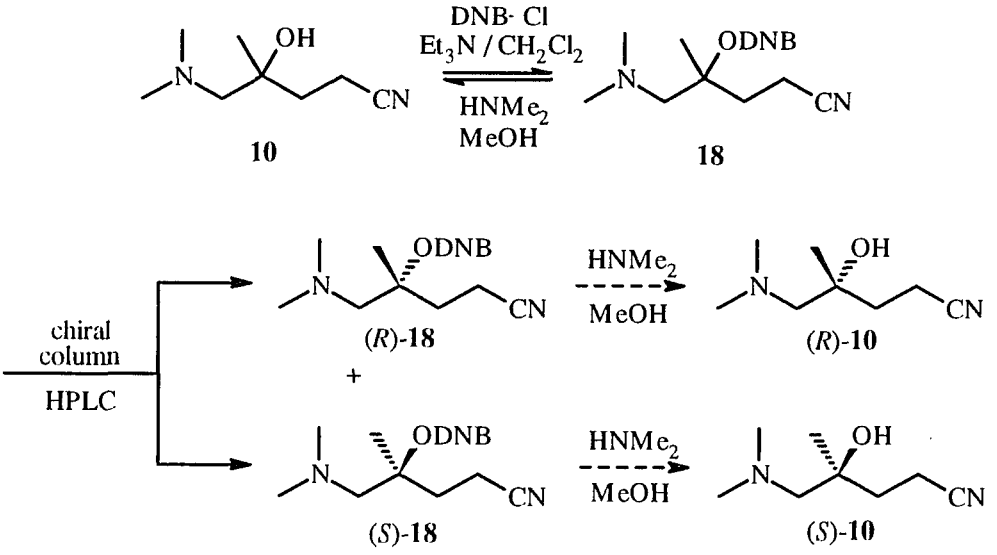
First, 3-methyl-3-buten-1-ol, **12**, was converted into 3-methyl-3-butenyl methanesulfonate, **16**, by treatment with methanesulfonyl chloride in dichloromethane at 0 °C in 98% yield. Then treatment of **16** with sodium cyanide in DMSO gave 4-methyl-4-pentenenitrile, **17**, in 77% yield. Epoxidation[86] of **17** with monoperoxyphthalic acid (MMPP), magnesium salt hexahydrate, gave (*RS*)-3-(1-methyloxiranyl)propanenitrile, **11**, in 84% yield. Compound **11** could also be obtained by epoxidation of **17** with *m*-chloroperoxybenzoic acid (*m*-CPBA).[87]

By comparison with *m*-CPBA, MMPP is non-shock-sensitive and non-deflagrating. Consequently, MMPP is much safer to use in both small- and large-scale operations. In addition, both MMPP and the oxidation byproduct, magnesium phthalate, are water-soluble thus easily removed by extraction after completion of the reaction.

(*RS*)-5-(*N,N*-Dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, was obtained by ring opening of (*RS*)-**11** with dimethylamine in methanol at room temperature in 90% yield. Compound **17** was identified by comparison of ^{1}H NMR and IR with literature.[88] Compound **11** was identified by comparison of ^{1}H NMR with literature.[87,89] Compound **10** was characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analysis.

II.2.2.2. Optical resolution of (*RS*)-5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, (*RS*)-10

(*RS*)-5-(*N,N*-Dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, was resolved as the 3,5-dinitrobenzoate esters **18** by HPLC using a chiral column (Scheme II.12). First, compound **10** was treated with 3,5-dinitrobenzoyl chloride in dichloromethane in the presence of triethylamine at 0 °C to give a racemic mixture of **18** in 100% yield. Then this racemic mixture was resolved by chiral column HPLC (CHIRALCEL OD column, 25 cm × 2 cm, J. T. Baker Inc., hexanes:ethanol = 6:1) to give two enantiomers (*R*)- and (*S*)-**18**.



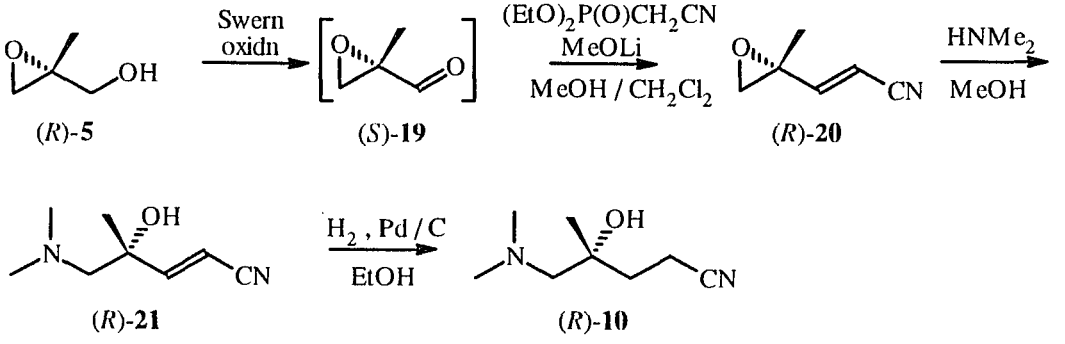
Scheme II.12

After the optical resolution of **18**, compounds (*R*)-**10** and (*S*)-**10** could be obtained by aminolysis of (*R*)-**18** and (*S*)-**18** respectively. We tried the aminolysis reaction with (*RS*)-**18**. Dimethylamine (2 equivalents) was bubbled into a solution of (*RS*)-**18** in methanol. The reaction was completed within half an hour, giving (*RS*)-**10** in 76% yield. (If we use (*R*)-**18** or (*S*)-**18**, we shall get (*R*)-**10** or (*S*)-**10**.)

We tried other methods to resolve compound **10**, such as (a) making quaternary ammonium salts with chiral acids (tartaric acid, dibenzoyl tartaric acid, 3-bromocamphor-9-sulfonic acid, *N*-acetyl-L-glutamic acid, mandelic acid, and malic acid) followed by recrystallization and (b) converting it into an ester with a chiral acid chloride ((*1S*)-(+)-camphanic chloride). These methods did not work.

II.2.2.3. Preparation of (*R*)- and (*S*)-5-(*N*,*N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, (*R*)- and (*S*)-10

Another route for preparation of (*R*)- and (*S*)-5-(*N*,*N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, is shown in Scheme II.13.



Scheme II.13

(*R*)-2-Methylglycidol, **5**, was converted into (*R*)-3-(1-methyloxiranyl)-2-propenenitrile, **20**, by Swern oxidation of **5** followed by Wittig reaction with diethyl cyanomethylphosphonate. The Swern oxidation reaction of **5** was conducted in dichloromethane at -50 to -60 °C (acetonitrile/acetone/dry ice bath) and 1.5 equivalents of oxalyl chloride and 3 equivalents of DMSO were needed for the completion of the reaction. After workup, (*S*)-(1-methyloxiranyl)formaldehyde, **19**, was obtained as a solution in dichloromethane. Compound **19** was too volatile to separate from dichloromethane. Therefore, the solution was used directly for the Wittig reaction. Diethyl cyanomethylphosphonate was treated with lithium methoxide in methanol to generate phosphonate carbanion. Then, the solution of **19** in dichloromethane was added. After stirring overnight at

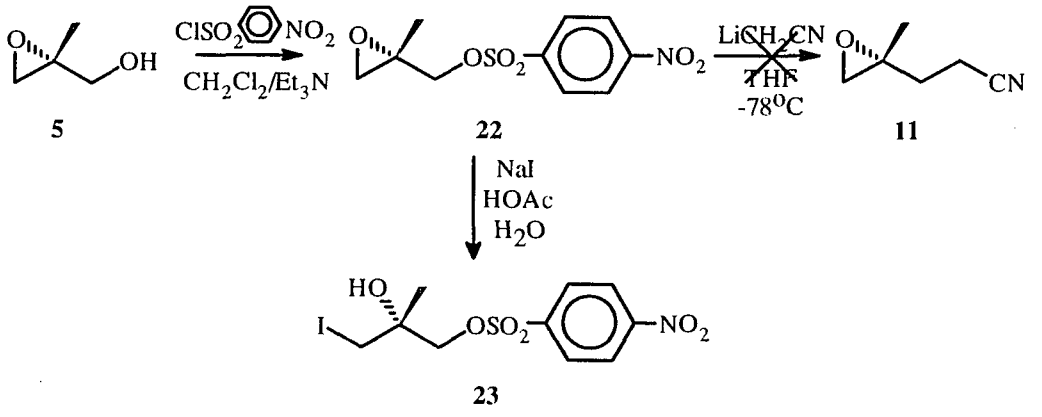
room temperature, **20** was isolated in 43% yield. Compounds (*R*)-**20** and (*S*)-**20** were prepared from (*R*)-**5** and (*S*)-**5** respectively and characterized by ^{1}H NMR and FT-IR.

Compounds (*R*)- and (*S*)-**20** were treated with dimethylamine in methanol to give (*R*)- and (*S*)-5-(*N*,*N*-dimethylamino)-4-hydroxy-4-methyl-2-pentenenitrile, **21**, respectively in 89% yield, which were characterized by ^{1}H NMR and FT-IR. Catalytic hydrogenation (10% Pd on charcoal) of (*R*)- and (*S*)-**21** in absolute ethanol at 25 psi and room temperature for 24 hours gave (*R*)- and (*S*)-5-(*N*,*N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, respectively in 87% yield.

By this route, compounds (*R*)- and (*S*)-**10** can be made from (*R*)- and (*S*)-**5** respectively. But, as discussed at the beginning of section II.2.1, both (*R*)- and (*S*)-**5** are not optically pure. After optical enrichment by making the 4-nitrobenzoate ester, **13**, followed by recrystallization, how to regenerate compound **5** is still a challenge.

II.2.2.4. Attempted preparation of (*R*)- and (*S*)-3-(1-methyloxiranyl) propanenitrile, (*R*)- and (*S*)-11

We tried to prepare (*R*)- and (*S*)-3-(1-methyloxiranyl)propanenitrile, **11**, from (*R*)- and (*S*)-**5**. As shown in Scheme II.14, compounds (*R*)- and (*S*)-**5** were converted into (*S*)- and (*R*)-(1-methyloxiranyl)methyl 4-nitrobenzenesulfonate, **22**, by reaction with 4-nitrobenzenesulfonyl chloride in dichloromethane in the presence of triethylamine at 0 °C.



Scheme II.14

Recrystallization of **22** from ethanol provided light yellow crystals. In order to measure the optical purity of **22** by Mosher's ester analysis,[82] compound **22** was converted into 2-hydroxy-3-iodo-2-methylpropyl 4-nitrobenzenesulfonate, **23**, by reaction with sodium iodide in acetic acid and water.[90] Crystals of **23** were obtained by recrystallization from methanol. Unfortunately, compound **23** could not be transformed into Mosher's ester because it is a tertiary alcohol. The conversion of **22** into **11** failed. Klunder et al[91] studied the reactivities of glycidyl arenesulfonate derivatives toward different organometallic reagents. In most cases studied, ring opening occurred. Johnson and Dutra[92] reported that tosylates and epoxy rings had similar reactivities toward lithium diorganocuprates. Therefore, compounds (*R*)- and (*S*)-**11** could not be prepared this way.

Compounds **22** and **23** were identified by ^1H NMR and verified by single-crystal X-ray analyses.[93,94]

II.2.2.5. Structural analysis of (*R*)-2-hydroxy-3-iodo-2-methylpropyl 4-nitrobenzenesulfonate, (*R*)-23

For compound (*R*)-**23** (Figure II.6),[94] we used PCMODEL[95] to calculate the eighty-one possible staggered conformations of the four torsion-angle sequence—IC-C-C-O-SO_2Ar. For the global minimum, those torsion angles are -58.9°, -55.2°, 169.3°, and -83.6°, respectively. In the crystal, they are -57.7 (4)°, -61.7 (4)°, 171.2 (2)°, and -73.0 (3)°, respectively. In this conformation, the I atom is on top of the benzene ring with nearly equal distances between the I atom and six C atoms of the benzene ring. The intramolecular distance [4.083 (2) Å] between I and the centroid of the aryl ring is almost equal to the sum (3.90 Å) of the van der Waals radius of I and the thickness of the benzene ring.[96] With respect to bond distances and angles, PCMODEL agrees approximately with the X-ray determination. Notable exceptions are the C1-C2, C7-C8, and C8-C9 bond lengths and the C7-C8-C9 and O5-N-O6 bond angles, as well as many values of the SO_3 group. The X-ray results reveal that the O2-C3 bond is 0.049 Å longer than the O1-C2 bond, which is consistent with OSO_2Ar being a better leaving group than the OH group. Molecules are linked in chains along the crystallographic **b** axis by weak intermolecular hydrogen bonds involving

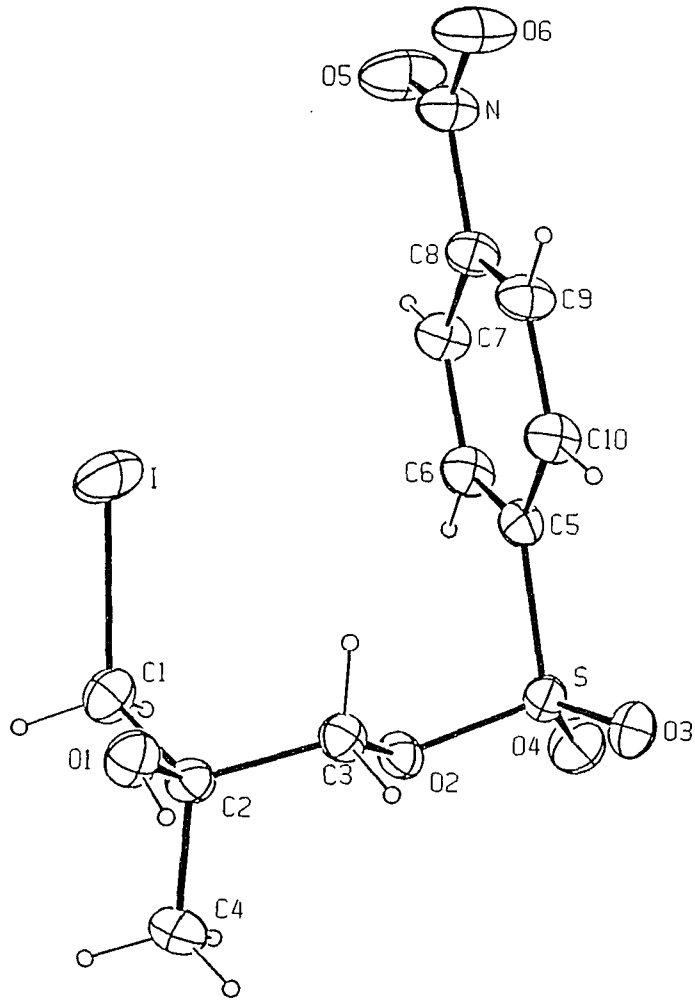
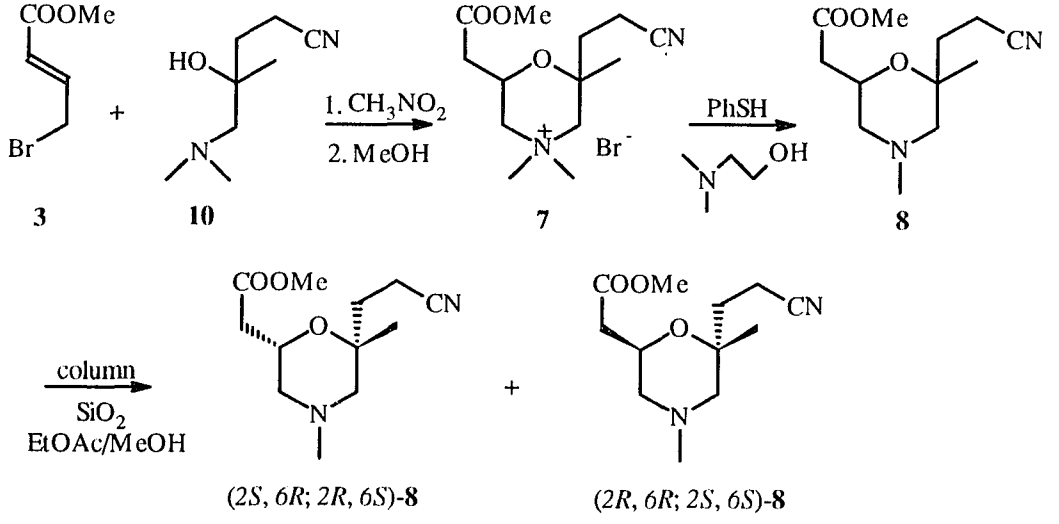


Figure II.6. ORTEP drawing of (*R*)-2-hydroxy-3-iodo-2-methylpropyl 4-nitrobenzenesulfonate

$O_{O\text{-}H}$ and $O_{S=O}$. The O1···O3 distance is 2.927 (4) Å and the O···H-O angle is 165 (4)°.

II.2.2.6. Syntheses of methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, 8

Methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**, were synthesized starting from (*RS*)-5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, as shown in Scheme II.15.



Scheme II.15

Racemic mixture of **10** reacted with 1 equivalent of methyl 4-bromo-2-butenoate in nitromethane at 50 to 60 °C for 3 hours. Then solvent was changed to methanol and refluxed for 18 hours. In nitromethane, condensation of **10** with **3** took place to produce acyclic quaternary amine

salt. In methanol, ring closure reaction occurred. The ring closure reaction cannot be carried out in THF, as in the reaction of **14**, due to the insolubility of the acyclic intermediate. Concentration of the reaction mixture followed by washing with diethyl ether and drying under vacuum gave crude product (*2S,6S;2R,6R;2S,6R;2R,6S*)-2-(2-cyanoethyl)-6-(methoxycarbonyl)methyl-2,4,4-trimethylmorpholinium bromide, **7**, in quantitative yield. This mixture of four stereoisomers could not be separated by normal-phase HPLC because they were quaternary amine salts. We tried reversed-phase HPLC to separate the two pairs of racemates, (*2R,6R;2S,6S*)-**7** and (*2S,6R;2R,6S*)-**7**, and we failed. The ^{1}H NMR spectrum of **7** was too complicated to interpret because it was a mixture of four stereoisomers. Therefore, the crude mixture of **7** was used for next reaction without further purification, separation, and characterization.

Crude **7** was treated with 3 equivalents of thiophenol in *N,N*-dimethylethanolamine[97] at 70 °C to give methyl (*2S,6S;2R,6R;2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**, which was separated by column chromatography to give two racemic mixtures, (*2S,6S;2R,6R*)-**8** as a solid and (*2S,6R;2R,6S*)-**8** as an oil, with a ratio of 3:4 and total yield of 25%. The low yield of the demethylation reaction may be caused mainly by the attack of thiophenol on the ester group of **7**.[98,99] The ratio of the two racemic mixtures indicates that the stereoselectivity of the ring closure reaction is not significant. The slight preference for (*2S*,6R;*2R,6S*)-**7** over (*2R,6R;2S,6S*)-**7** may be attributed to chair transition structures in the ring

closure reaction (Figure II.7). The 2-cyanoethyl group is bigger than methyl group and tends to be *cis* with (methoxycarbonyl)methyl so that both cyanoethyl and (methoxycarbonyl)methyl can adopt equatorial positions. Crystals of (*2S,6S;2R,6R*)-**8** for X-ray analysis were obtained by recrystallization from hexanes. Compounds (*2S,6S;2R,6R*)-**8** and (*2S,6R;2R,6S*)-**8** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy, and elemental analyses.

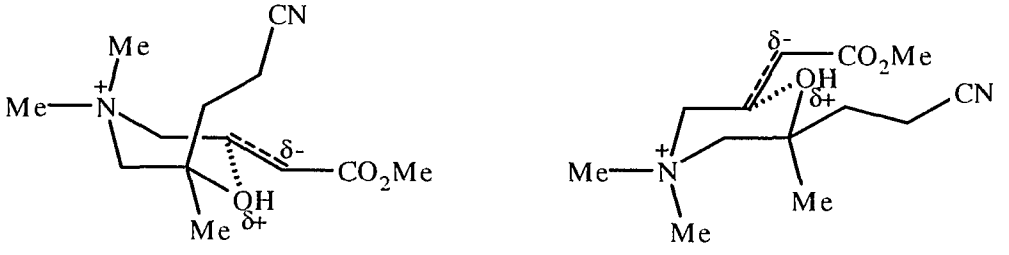


Figure II.7. Transition structures for ring closure reaction of **10**

The structure of (*2S,6S;2R,6R*)-**8** was verified by single-crystal X-ray analysis (Figure II.8).[100] The morpholine ring is in a chair conformation. The methoxycarbonylmethyl and methyl on C3 are *cis*; both occupy equatorial positions, as does the *N*-methyl group. We used PCMODEL[95] to calculate the eighty-one possible staggered conformations of the four torsion-angle sequence—NC-CH_2-CH_2-C-O and O-CH-CH_2-C(O)-OCH_3. For the global minimum, which was also found by GMMX,[101] those torsion angles are 179.2°, -50.5°, -60.0°, and 140.2°, respectively. In the crystal, they are

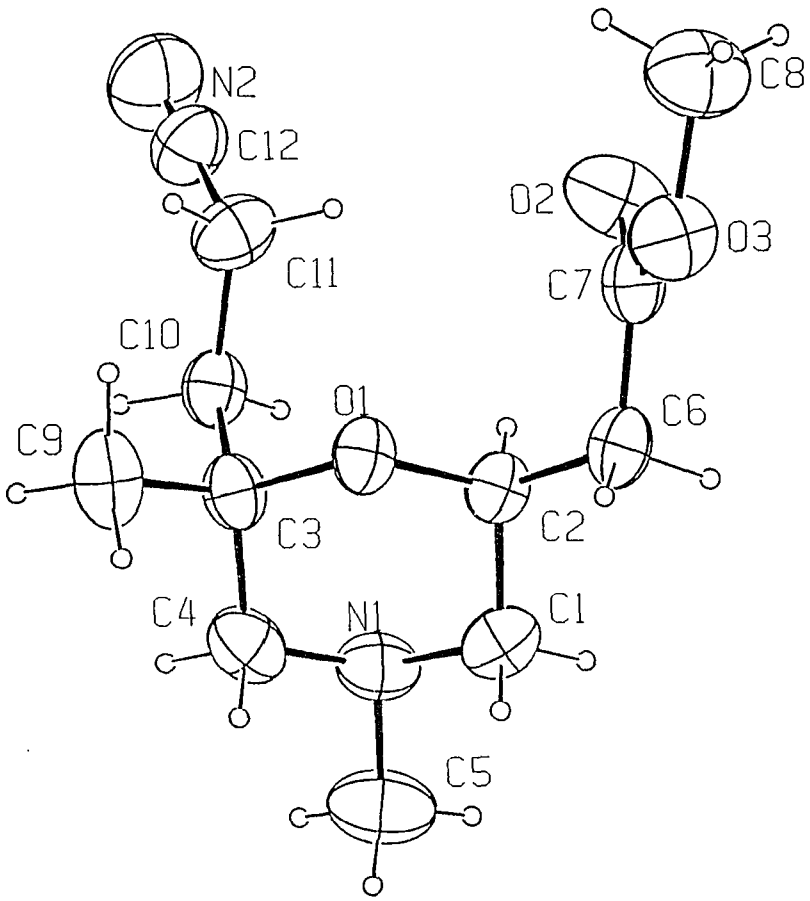
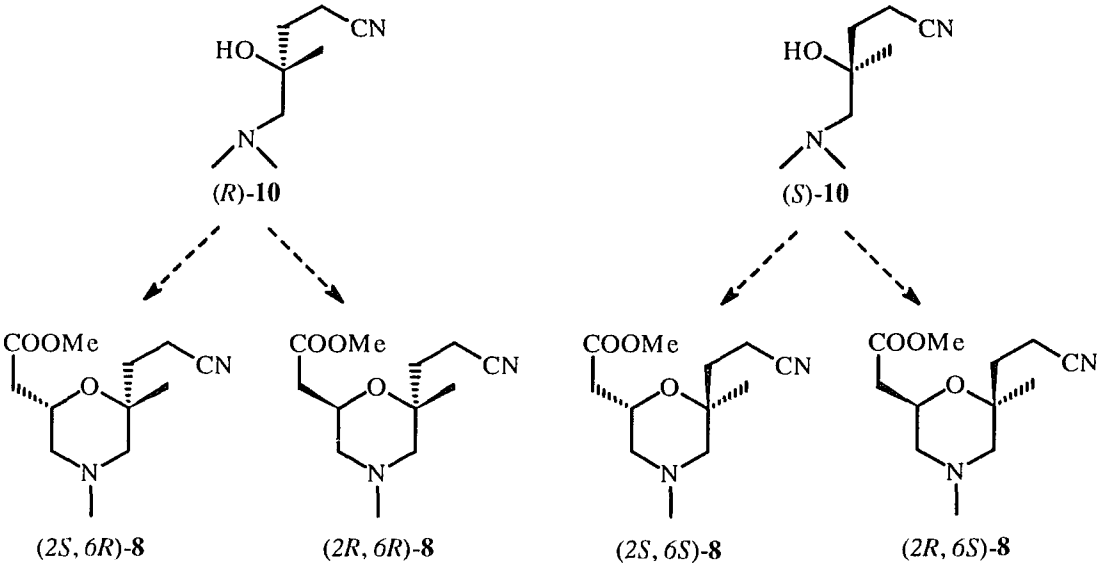


Figure II.8. ORTEP drawing of (*2S,6S;2R,6R*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate

-170.0 (1)°, -45.9 (2)°, -71.6 (2)°, and 142.8 (1)°, respectively. With respect to bond distances and angles, PCMODEL also agrees with the X-ray determination.

In this synthetic route, if we start with (*R*)- or (*S*)-5-(*N*,*N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, which can be obtained by optical resolution, in place of (*RS*)-**10**, we shall be able to get the four stereoisomers of **8** separated (Scheme II.16).



Scheme II.16

II.2.3. Summary

(*2R,6R*)-, (*2S,6S*)-, (*2R,6S*)-, and (*2S,6R*)-6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, target molecule **II**, and methyl

(*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethyl-morpholinyl]acetate **8** were synthesized. (*RS*)-5-(*N,N*-Dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, was resolved.

The method for synthesizing target molecule **II** in section II.2.1 can be further extended for synthesizing a series of other inhibitors by changing hydroxy group of **2**, **1** or target molecule **II** to other groups, which will be discussed in Chapter IV. Target molecule **III** can also be approached by this strategy so that the ultimate goal of this project can be achieved.

By synthetic route in section II.2.2, we got compound **8**, from which target molecule **III** can be derived. But by this route we cannot get other inhibitors which can be obtained by the extension of the method for synthesizing target molecule **II** (see Chapter IV).

II.3. EXPERIMENTAL

II.3.1. General procedures

Uncorrected melting points were measured on an Electrothermal melting point apparatus. ^{1}H NMR spectra were recorded with a Bruker AMX 500, AM 400, AC 200, or AC 100 FT-NMR spectrometer at 500, 400, 200, or 100 MHz, respectively. ^{13}C NMR spectra were recorded with a Bruker AC 200 FT-NMR spectrometer at 50 MHz. Unless noted otherwise, all NMR spectra were recorded in $CDCl_3$. Proton chemical shifts are expressed in parts per million (ppm) down field from internal tetramethylsilane (TMS); coupling constants that were verified using PANIC

(Parameter Adjustment in NMR by Iteration Calculation) are listed as J; observed coupling constants not verified are listed as J_{app}; all coupling constants are reported in Hz. ^{13}C chemical shifts are also expressed in ppm relative to the solvent chemical shift. Assignments of the ^{1}H and ^{13}C NMR signals were made by comparison with similar known compounds and using DEPT, 2D ^{13}C-^{1}H correlation, and 2D ^{1}H COSY experiments. Infrared spectra were recorded on a Perkin Elmer 1760X FT-IR spectrometer as thin films on KBr cells and are reported in cm^{-1}. Mass spectra were obtained with a Hewlett-Packard 5971A GC-MS. FAB MS samples were prepared by suspending in glycerol and the spectra were recorded on a Finnigan TSQ 70. Elemental analyses were performed by Oneida Research Services of Whitesboro, New York. The optical rotations were recorded in a 3.5×10 mm or 10×100 mm cell with a JASCO DIP-370 digital polarimeter.

Unless otherwise noted, materials were obtained from commercial sources and used without further purification. Tetrahydrofuran (THF) was distilled from potassium. Diethyl ether was distilled from sodium-potassium alloy. Dimethyl sulfoxide (DMSO) was distilled from CaH_2 under reduced pressure and stored over Linde Molecular Sieves Type 3A in a sealed brown bottle. Triethylamine was distilled from CaH_2 and stored over Linde Molecular Sieves Type 3A. Dichloromethane was purified by shaking with concd H_2SO_4, washing with H_2O and satd brine, drying with CaH_2, distilling, and storing over 4A Molecular Sieves. Methanol was distilled over a small amount of Mg. 3,5-Dinitrobenzoyl chloride, 4-nitrobenzoyl chloride,

and 4-nitrobenzenesulfonyl chloride were recrystallized from hexanes. Thionyl chloride was distilled before use. Methyl 4-bromo-2-butenoate was purified by vacuum distillation.

The organic solutions were dried ($MgSO_4$) and concentrated by rotary evaporation unless otherwise noted.

II.3.2. Procedures

(*R*)-(1-Methyloxiranyl)methyl 4-nitrobenzoate ((*R*)-13). A solution of 4-nitrobenzoyl chloride (116 mmol, 21.6 g) in CH_2Cl_2 (30 mL) was slowly added to a stirred mixture of (*S*)-2-methylglycidol (110 mmol, 9.98 g, 97% purity) and Et_3N (124 mmol, 12.6 g) in CH_2Cl_2 (90 mL) at 0 °C (ice-water bath). The reaction mixture was stirred for additional 2 h at 0 °C. The reaction mixture was diluted with cold CH_2Cl_2 (60 mL) and filtered with suction to remove precipitated ammonium salt, which then was washed with cold CH_2Cl_2 (3 × 15 mL). The mother liquor was washed with cold 5% HCl (60 mL), cold H_2O (2 × 40 mL), and dried. Concentration of the solution gave 25.54 g of a light yellow solid. Recrystallization from EtOH (300 mL) gave 19.81 g of light yellow crystals. The crystals were dissolved in Et_2O (250 mL) while refluxing and then the solution was cooled slowly to 10 °C, giving 15.65 g of crystals, which were then recrystallized from $(i\text{-}Pr)_2O$ (130 mL) to give 12.8 g (49 %, ee > 97%) of (*R*)-**13** (mp 87.5-89.0 °C, Lit.[83] 85.5-86.5 °C).

^1H NMR (200 MHz; Lit.[83]): 1.49 (s, 3 H, CH_3-), 2.76 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.6), 2.87 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.5), 4.24 (d, 1 H, *H*-

CHOCOAr, J_{app} = 11.9), 4.58 (d, 1 H, *H*-CHOCOAr, J_{app} = 12.0), 8.21-8.34 (m, 4 H, Bz). $[\alpha]^{23}_{D}$ -5.85° (c 2.035, $CHCl_3$, 10 × 100 mm cell. Lit.[83] -5.87°). (*S*)-**13** was prepared from (*R*)-2-methylglycidol the same way as (*R*)-**13**. mp 87.5-89.0 °C. $[\alpha]^{23}_{D}$ +5.83° (c 1.989, $CHCl_3$ 10 × 100 mm cell).

(*R*)-3-Methylamino-2-methylpropane-1,2-diol ((*R*)-4). A solution of (*R*)-**13** (100 mmol, 23.7 g) in MeOH (200 mL) was added dropwise to a solution of H_2NMe (0.6 mol, 18.6 g) in MeOH (60 mL). The mixture was stirred for 3 h. The precipitate was removed by filtration. The solution was placed in a refrigerator (0 °C) for 0.5 h then in a freezer for 1 h. Again, the precipitate was removed by filtration. The solution was concentrated until a large amount of precipitate appeared and then placed in a freezer for 0.5 h. Again, the precipitate was removed by filtration. The solution was concentrated and then placed under vacuum. The residue was distilled under vacuum affording 8.92 g (75%) of (*R*)-**4** as a colorless oil (bp 80 °C/0.2 Torr, 160 °C/17 Torr).

^{1}H NMR (200 MHz): 1.10 (s, 3 H, C*H*$_3$-C), 2.44 (s, 3 H, C*H*$_3$-N), 2.66 (d, 1 H, *H*-CHN, J_{app} = 12.1), 2.74 (dd, 1 H, *H*-CHN, J_{app} = 12.2, 1.4), 3.48 (dd, 1 H, *H*-CHOH, J_{app} = 11.2, 1.4), 3.65 (d, 1 H, *H*-CHOH, J_{app} = *11.0).* ^{13}C NMR: 23.2 (CH_3-C), 36.9 (CH_3-N), 60.9 (-CH_2-N), 70.9 (*C*-OH), 71.2 (-CH_2OH). IR: 3321 (OH), 1055 (C-N). MS *m/e* (relative intensity): 88 (11.4), 75 (3.6), 70 (7.3), 58 (10.4), 57 (4.3), 45 (5.1), 44 (100), 43 (10.2), 42 (11.1). Anal. Calcd for $C_5H_{13}NO_2$: C, 50.42; H, 10.92; N, 11.76. Found: C, 50.14; H, 11.02; N, 11.57.

(*S*)-**4** was prepared from (*R*)-**13** the same way as (*R*)-**4**. Anal. Found: C, 50.29; H, 10.89; N, 11.56.

(*R*)-Methyl 4-[methyl-(2,3-dihydroxy-2-methylpropyl)amino]-2-butenoate ((*R*)-14). A solution of methyl 4-bromo-2-butenoate (40.34 mmol, 7.22 g) in THF (30 mL) was added to a mixture of (*R*)-**4** (40.34 mmol, 4.80 g) and K_2CO_3 (54 mmol, 7.4 g) in THF (50 mL). The reaction mixture was stirred overnight and then filtered. The solution was concentrated and then placed under vacuum. Dry Et_2O (40 mL) was added and the precipitate was removed by filtration. Concentration of the solution gave 7.26 g (83%) of a light yellow oil, which was used for next reaction without further purification.

^{1}H NMR (200 MHz): 1.07 (s, 3 H, C*H*$_3$-C), 2.38 (s, 3 H, C*H*$_3$-N), 2.49 (d, 1 H, *H*-CHC(OH)(CH$_3$)CH$_2$OH, J_{app} = 13.8), 2.62 (d, 1 H, *H*-CHC(OH)(CH$_3$)CH$_2$OH, J_{app} = 13.8), 3.13-3.63 (m, 4 H, H_2C-CH=CH-, H_2C-OH), 3.73 (s, 3 H, C*H*$_3$-O), 5.90-6.00 (m, 1 H, *H*C-CO$_2$CH$_3$), 6.85-6.99 (m, 1 H, *H*C=CH-CO$_2$CH$_3$). ^{13}C NMR (50 MHz): 23.7 (*C*H$_3$-C), 45.0 (*C*H$_3$-N), 51.6 (*C*H$_3$O), 60.4 (*C*H$_2$-CH=CH), 65.4 (N-*C*H$_2$-COH), 70.2 (*C*H$_2$-OH), 71.5 (*C*-OH), 123.2 (=*C*H-CO$_2$CH$_3$), 144.9 (*C*H=CHCO$_2$CH$_3$), 166.4 (*C*O$_2$CH$_3$). IR: 3425 (OH), 1724 (C=O), 1660 (C=C). MS, FAB, *m/e*: 218 (M^++1).

(*S*)-**14** was prepared from (*S*)-**17** the same way as (*R*)-**14**.

Methyl (*2S,6R*)-2-(4,6-dimethyl-6-hydroxymethylmorpholinyl) acetate ((*2S,6R*)-2) and methyl (*2R,6R*)-2-(4,6-dimethyl-6-hydroxymethylmorpholinyl) acetate ((*2R,6R*)-2). DBU (2.5 mL, 16.4 mmol) was

added to a solution of crude (*R*)-**14** (33.4 mmol, 7.26 g) in THF (500 mL). The reaction mixture was stirred at rt for 24 h. Concentration of the solution gave a brown liquid, which was purified by column chromatography (SiO_2, 70-230 mesh, sample:SiO_2 = 1:25, EtOAc:MeOH = 100:5), yielding a mixture of two diastereomers. The diastereomers were separated by column chromatography (SiO_2, 230-400 mesh, sample:SiO_2 = 1:70, hexanes:CH_2Cl_2:EtOH = 100:100:30), giving (*2S,6R*)-**2** (2.18 g, 30%) and (*2R,6R*)-**2** (0.70 g, 9.6%).

(*2S,6R*)-**2**:

^{1}H NMR (500 MHz): 1.11 (s, 3 H, C*H*$_3$-C6), 1.74 (dd, 1 H, *H*(ax)-C3, *J* = 10.87, 10.73), 1.94 (d, 1 H, *H*(ax)-C5, *J* = 11.83), 2.20 (s, 3 H, C*H*$_3$-N), 2.37 (dd, 1 H, *H*-CHCO$_2$CH$_3$, *J* = 16.35, 4.06), 2.49 (dd, 1 H, *H*-CHCO$_2$CH$_3$, *J* = 16.35, 8.93), 2.65 (d, 1 H, *H*(eq)-C5, *J* = 11.83), 2.70 (d, 1 H, *H*(eq)-C3, *J* = 10.87), 3.40 (d, 1 H, *H*-CHOH, *J* = 11.58), 3.70 (s, 3 H, C*H*$_3$-O), 4.20 (d, 1 H, *H*-CHOH, *J* = 11.58), 4.29-4.37 (m, *H*-C2). ^{13}C NMR: 23.6 (*C*H$_3$-C), 38.2 (*C*H$_2$-CO$_2$CH$_3$), 45.9 (*C*H$_3$-N), 51.7 (*C*H$_3$-O), 58.8 (*C*3), 61.3 (*C*5), 65.8 (*C*H$_2$-OH), 66.6 (*C*2), 73.5 (*C*6), 171.5 (*C*=O). IR: 3506 (OH), 1741 (C=O). MS *m/e* (relative intensity): 217 (19.5), 186 (54.5), 144 (13.7), 143 (26.5), 142 (31.6), 128 (17.6), 114 (10.5), 98 (18.4), 70 (25.1), 59 (16.9), 58 (17.6), 57 (21.5), 44 (47.9), 43 (100), 42 (60.4), 41 (27.0). $[\alpha]^{23}_D$, -20.2° (c 9.95, $CHCl_3$, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.30; H, 8.76; N, 6.45. Found: C, 54.98; H, 8.73; N, 6.47.

(*2R,6R*)-**2**:

^{1}H NMR (500 MHz): 1.29 (s, 3 H, CH_3-C6), 1.68 (dd, 1 H, *H*(ax)-C3, *J* = 11.08, 10.96), 2.09 (d, 1 H, *H*(ax)-C5, *J* = 10.95), 2.24 (s, 3 H, CH_3-N), 2.39 (dd, 1 H, *H*-CHCO_2CH_3, *J* =15.30, 5.74), 2.42 (d, 1 H, *H*(eq)-C5, *J* = 10.95), 2.48 (dd, 1 H, *H*-CHCO_2CH_3, *J* = 15.30, 7.11), 2.75 (d, 1 H, *H*(eq)-C3, *J* = 11.08), 3.33 (d, 1 H, *H*-CHOH, *J* = 11.23), 3.47 (d, 1 H, *H*-CHOH, *J* = 11.23), 3.69 (s, 3 H, CH_3-O), 4.21-4.27 (m, 1 H, *H*-C2). ^{13}C NMR: 18.7 (*C*H_3-C), 38.9 (CH_2-CO_2CH_3), 46.4 (*C*H_3-N), 51.6 (*C*H_3-O), 59.3 (*C*3), 59.7 (*C*5), 66.5 (*C*2), 69.2 (*C*H_2-OH), 74.3 (*C*6), 171.1 (*C*=O). IR: 3452 (OH), 1741 (C=O). MS *m/e* (relative intensity): 217 (21.1), 186 (56.2), 144 (16.1), 143 (26.5), 142 (37.4), 128 (15.0), 114 (13.3), 98 (17.7), 84 (84.8), 70 (26.0), 59 (15.6), 58 (12.7), 57 (19.2), 44 (48.3), 43 (100), 42 (40.7), 41 (15.3). $[\alpha]^{23}_D$, +2.24° (c 11.6, $CHCl_3$, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.30; H, 8.76; N, 6.45. Found: C, 54.90; H, 8.45; N, 6.33.

(*2R,6S*)-**2**, (*2S,6S*)-**2** were prepared from (*S*)-**14** the same way as (*2S,6R*)-**2** and (*2R,6R*)-**2**.

(*2R,6S*)-**2**:

$[\alpha]^{23}_D$ +20.6° (c 5.93, $CHCl_3$, 3.5 × 10 mm cell). Anal. Found: C, 54.98; H, 8.45; N, 6.46.

(*2S,6S*)-**2**:

$[\alpha]^{23}_D$ -2.26° (c 9.28, $CHCl_3$, 3.5 × 10 mm cell) . Anal. Found: C, 54.92; H, 8.50; N, 6.34.

(*2R,6S*)-2-Hydroxymethyl-6-methoxycarbonylmethyl-2,4,4-trimethylmorpholinium iodide ((*2R,6S*)-1). To a solution of (*2S,6R*)-**2**

(0.868 g, 4 mmol) in dry Et_2O (30 mL) was added CH_3I (5 mL, 80 mmol). The mixture was placed in the dark and stirred for 3 d. The solution was decanted and the precipitate was washed with dry Et_2O (3 × 5 mL). The yellow paste was dried under vacuum to give 1.06 g (74%) of a light yellow solid which was used for the next reaction without further purification. Crystals for X-ray analysis were obtained by recrystallization from MeOH by vapor diffusion with Et_2O.

(*2R,6S*)-**1** (mp 136.5-140 °C, dec.):

^{1}H NMR (400 MHz, CD_3OD, TMS): 1.27 (s, 3 H, C*H*$_3$-C2), 2.60 (dd, 1 H, *H*-$CHCO_2CH_3$, J = 16.13, 7.08), 2.65 (dd, 1 H, *H*-$CHCO_2CH_3$, J = 16.13, 5.04), 3.13 (d, 1 H, *H*(ax)-C3, J = 13.39), 3.26 (dd, 1 H, *H*(ax)-C5, J = 11.91, 12.82), 3.30 (s, 3 H, C*H*$_3$(eq)-N), 3.36 (s, 3 H, C*H*$_3$(ax)-N), 3.56 (d, 1 H, *H*-CHOH, J = 11.69), 3.63 (ddd, 1H, *H*(eq)-C5, J = 12.82, 1.56, 1.49), 3.71 (s, 3 H, C*H*$_3$O), 3.83 (dd, *H*(eq)-C3, J = 13.39, 1.56), 3.95 (d, 1 H, *H*-CHOH, J =11.69), 4.63-4.70 (m, 1 H, *H*-C6). ^{13}C NMR (CD_3OD): 26.94 (*C*H$_3$-C2), 38.31 (*C*H$_2$-CO_2CH_3), 51.52 (*C*H$_3$(ax)-N), 52.56 (*C*H$_3$O), 59.53 (*C*H$_3$(eq)-N), 63.74 (C3, C6), 63.96 (C5), 64.10 (*C*H$_2$OH), 74.98 (C2), 171.72 (*C*O$_2$CH$_3$). IR: 3346 (OH), 1734 (C=O), 1058 (C-O-C). MS, FAB, *m/e*: 232 (M-I$^-$). $[\alpha]^{22}_D$ -21.3° (c 7.10, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{11}H_{22}NO_4I$: C, 36.77; H, 6.13; N, 3.90. Found: C, 36.64; H, 6.02; N, 3.87.

(*2R,6R*)-**1**, (*2S,6S*)-**1**, and (*2S,6R*)-**1** were prepared the same way as (*2R,6S*)-**1** from (*2R,6R*)-**2**, (*2S,6S*)-**2**, and (*2R,6S*)-**2** respectively.

(*2R,6R*)-**1** (mp 136.2-140 °C, dec.):

^{1}H NMR (400 MHz, CD_3OD, TMS): 1.45 (s, 3 H, CH_3-C2), 2.63 (dd, 1 H, *H*-$CHCO_2CH_3$, *J* = 15.38, 8.41), 2.67 (dd, 1 H, *H*-$CHCO_2CH_3$, *J* =15.38, 4.91), 3.20 (dd, 1 H, *H*(ax)-C5, *J* = 12.05, 11.74), 3.31 (s, 3 H, CH_3(eq)-N), 3.34 (d, 1 H, *H*-CHOH, *J* = 11.71), 3.43 (s, 3 H, CH_3(ax)-N), 3.45 (d, 1 H, *H*(ax)-C3, *J* =13.74), 3.46 (d, 1 H, *H*-CHOH, *J* = 11.71), 3.57 (dd, 1 H, *H*(eq)-C3, *J* = 13.74, 1.84), 3.64 (ddd, 1 H, *H*(eq)-C5, *J* = 11.74, 2.18, 1.84), 3.71 (s, 3 H, CH_3O), 4.61-4.68 (m, 1 H, *H*-C6). ^{13}C NMR (CD_3OD): 19.93 (CH_3-C2), 38.13 ($CH_2CO_2CH_3$), 51.90 (CH_3(ax)-N), 52.49 (CH_3O), 59.74 (CH_3(eq)-N), 63.21 (*C*6), 64.34 (*C*5), 65.39 (*C*3), 69.92 (CH_2OH), 74.77 (*C*2), 171.62 (CO_2CH_3). IR: 3359 (OH), 1735 (C=O), 1066 (C-O-C). MS, FAB, *m/e*: 232 (M-I$^-$). $[\alpha]^{22}_D$ +15.1° (c 9.90, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{11}H_{22}NO_4I$: C, 36.77; H, 6.13; N, 3.90. Found: C, 36.67; H, 6.08; N, 3.85.

(*2S,6S*)-**1**:

$[\alpha]^{22}_D$ -14.8° (c 9.25, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 36.76; H, 6.08; N, 3.84.

(*2S,6R*)-**1**:

$[\alpha]^{22}_D$ +21.5° (c 6.40, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 36.66; H, 6.05; N, 3.84.

(*2R,6S*)-6-Carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethyl morpholinium ((*2R,6S*)-II). A solution of (*2R,6S*)-**1** (0.95 g, ~ 2.6 mmol) in 0.1 M NaOH (26 mL, 2.6 mmol) was stirred at rt overnight. The reaction mixture was concentrated by rotary evaporation, then dried under vacuum. The residual solid was dissolved in CH_3OH (30 mL) and filtered. The liquid

was concentrated by rotary evaporation and dried under vacuum. The resulting light yellow solid was dissolved in a minimum amount of CH_3OH, and acetone (~ 40 mL) was added. The solution was decanted and the precipitate was dried under vacuum. The solid obtained was dissolved in CH_3OH (80 mL). Then acetone (900 mL) was added. The solution was left open to the air on the bench, yielding 0.44 g (67%) of colorless crystals ($C_{10}H_{19}NO_4 \cdot 2H_2O$).

(*2R,6S*)-**II** (mp 230-231 °C):

^1H NMR (400 MHz, CD_3OD, TMS): 1.26 (s, 3 H, C*H*$_3$-C2), 2.30 (dd, 1 H, *H*-$CHCO_2CH_3$, J = 15.34, 6.44), 2.46 (dd, 1 H, *H*-$CHCO_2CH_3$, J = 15.34, 6.61), 3.06 (d, 1 H, *H*(ax)-C3, J = 13.40), 3.11 (dd, 1 H, *H*(ax)-C5, J = 12.55, 11.79), 3.23 (s, 3 H, C*H*$_3$(eq)-N), 3.30 (s, 3 H, C*H*$_3$(ax)-N), 3.56 (ddd, 1 H, *H*(eq)-C5, J = 12.55, 1.79, 1.68), 3.64 (d, 1 H, *H*-CHOH, J = 11.80), 3.75 (dd, 1 H, *H*(eq)-C3, J = 13.40, 1.79), 3.87 (d, 1 H, *H*-CHOH, J = 11.80), 4.49-4.57 (m, 1 H, *H*-C6). ^{13}C NMR (CD_3OD): 26.84 (*C*H$_3$-C2), 42.21 (*C*H$_2$CO$_2$CH$_3$), 51.24 (*C*H$_3$(eq)-N), 59.25 (*C*H$_3$(ax)-N), 63.89 (*C*H$_2$OH, C5), 64.92 (*C*6), 64.99 (*C*3), 74.65 (*C*2), 176.94 (*C*O$_2^-$). IR: 3387 (OH), 1586 (C=O), 1061 (C-O-C). MS, FAB, *m/e*: 218 (M^++1). $[\alpha]^{22}_D$ -23.1° (c 6.50, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4 \cdot 2H_2O$: C, 47.43; H, 9.09; N, 5.53. Found: C, 47.78; H, 8.93; N, 5.46.

(*2R,6R*)-**II**, (*2S,6S*)-**II**, and (*2S,6R*)-**II** were prepared the same way as (*2R,6S*)-**II** from (*2R,6R*)-**1**, (*2S,6S*)-**1**, (*2S,6R*)-**1** respectively.

(*2R,6R*)-**II** (mp 235.5-236.5 °C):

^{1}H NMR (400 MHz, CD_3OD, TMS): 1.42 (s, 3 H, C*H*$_3$-C2), 2.34 (dd, 1 H, *H*-$CHCO_2CH_3$, *J* = 14.82, 6.43), 2.45 (dd, 1 H, *H*-$CHCO_2CH_3$, *J* = 14.82, 6.26), 3.09 (dd, 1 H, *H*(ax)-C5, *J* = 12.26, 11.26),3.24 (s, 3 H, C*H*$_3$(eq)-N), 3.30 (d, 1 H, *H*(ax)-C3, *J* = 11.90), 3.35 (s, 3 H, C*H*$_3$(ax)-N), 3.42 (s, 2 H, C*H*$_2$OH), 3.46 (d, 1 H, *H*(eq)-C3, *J* = 11.90), 3.57 (d, 1 H, *H*(eq)-C5, *J* = 12.26), 4.51 (m, 1 H, *H*-C6). ^{13}C NMR (CD_3OD): 20.00 (*C*H_3-C2), 42.36 (*C*$H_2CO_2CH_3$), 51.65 (*C*H_3(eq)-N), 59.53 (*C*H_3(ax)-N), 64.48 (*C*6), 65.23 (*C*5), 65.43 (*C*3), 70.06 (CH_2OH), 74.44 (*C*2), 177.09 (*C*O_2^-). IR: 3583 (OH), 1587 (C=O), 1078 (C-O-C). MS, FAB, *m/e*: 218 (M^++1). $[\alpha]^{22}_D$ +15.6° (c 4.8, MeOH, 3.5 × 10 mm cell). Anal. Calcd for $C_{10}H_{19}NO_4 \cdot 2H_2O$: C, 47.43; H, 9.09; N, 5.53. Found: C, 47.31; H, 8.83; N, 5.47.

(*2S,6S*)-**II**:

$[\alpha]^{22}_D$ -16.0° (c 5.00, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 47.29; H, 8.77; N, 5.57.

(*2S,6R*)-**II**:

$[\alpha]^{22}_D$ +23.1° (c 5.40, MeOH, 3.5 × 10 mm cell). Anal. Found: C, 47.05; H, 8.89; N, 5.34.

3-Methyl-3-butenyl methanesulfonate (16). A solution of methanesulfonyl chloride (0.42 mol, 49.0 g) dissolved in CH_2Cl_2 (100 mL) was added dropwise to a stirred solution of 3-methyl-3-buten-1-ol (0.40 mol, 35.50 g) and Et_3N (0.60 mol, 60.6 g) in CH_2Cl_2 (400 mL) at 0 °C (ice-water bath). After completion of the addition, the reaction mixture was stirred for 2 h at 0 °C. The reaction mixture was diluted with cold CH_2Cl_2 (200 mL) and

filtered with suction to remove precipitated ammonium salt which was then washed with cold CH_2Cl_2 (100 mL). The mother liquor was washed with cold 5% HCl (200 mL), cold H_2O (2 × 150 mL), and dried. Concentration of the solution gave 64.29 g (98%) of a brown oil, which was used without further purification.

^{1}H NMR (200 MHz; Lit.[102]): 1.78 (s, 3 H, CH_3-C=C), 2.47 (t, 2 H, -CH_2-C=C, J_{app} = 6.8), 3.01 (s, 3 H, CH_3-S), 4.33 (t, 2 H, -CH_2-OS, J_{app} = 6.9), 4.84 (m, 2 H, CH_2=C).

4-Methyl-4-pentenenitrile (17). Under N_2, a solution of **16** (0.39 mol, 64.29 g) in DMSO (100 mL) was added dropwise to a stirred slurry of NaCN (0.60 mol, 31.9 g) in DMSO (250 mL) at 80 °C. After the completion of the addition, the reaction mixture was stirred for 5 h at 80 °C, cooled, poured into ice-H_2O (800 mL), and extracted with Et_2O (4 × 800 mL). The combined extracts were washed with satd brine (3 × 800 mL) and dried. Concentration of the soluton gave a yellow oil. Vacuum distillation afforded 28.68 g (77%) of a colorless oil (bp 65-66 °C/17 Torr; Lit.[88] 50-52 °C/12 Torr).

^{1}H NMR (200 MHz; Lit.[88]): 1.77 (s, 3 H, CH_3-), 2.30-2.55 (m, 4 H, CH_2CH_2CN), 4.80-4.88 (m, 2 H, CH_2=C). ^{13}C NMR: 15.5 (*C*2), 21.7 (*C*H$_3$), 32.7 (*C*3), 112.0 (*C*5), 119.1 (*C*N), 141.4 (**C**4). IR (Lit.[88]): 3081 (olefinic C-H stretch), 2247 (C≡N), 1653 (C=C stretch), 898 (olefinic C-H bend).

(*RS*)-3-(1-Methyloxiranyl)propanenitrile (11). A solution of monoperoxyphthalic acid, magnesium salt hexahydrate (70 mmol, 43.30 g, 80% purity) in H_2O (200 mL) was added dropwise to a solution of **17** (100

mmol, 9.50 g) in i-PrOH (60 mL). The mixture was stirred for 5 h at rt. After addition of H_2O (100 mL), the reaction mixture was extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were washed with 1 M Na_2SO_3 (100 mL), 10% Na_2CO_3 (100 mL), H_2O (2 × 200 mL), dried, and concentrated affording a colorless oil. Vacuum distillation yielded 9.32 g (84%) of a colorless oil (bp 83-84 °C/17 Torr; Lit.[89] 110 °C/25 Torr).

^{1}H NMR (200 MHz, Lit.[87]): 1.36 (s, 3 H, C*H*$_3$-), 1.95 (t, 2 H, -C*H*$_2$CH$_2$CN, J_{app} = 7.4), 2.43 (t, 2 H, C*H*$_2$CN, J_{app} = 7.6), 2.64 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.4), 2.72 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.3). ^{1}H NMR (200 MHz, C_6D_6; Lit.[89]): 0.84 (s, 3 H, C*H*$_3$-), 1.20-1.64 (m, 4 H, C*H*$_2$C*H*$_2$CN), 2.07 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7), 2.13 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7). ^{13}C NMR: 12.4 (*C*2), 20.1 (*C*H$_3$), 31.6 (*C*3), 52.8 (*C*H$_2$=oxirane), 54.8 (*C*=oxirane), 119.0 (*C*N). IR: 3043 (oxirane), 2250 (C≡N).

(*RS*)-5-(*N,N*-Dimethylamino)-4-hydroxy-4-methylpentanenitrile (10). A solution of **11** (52 mmol, 5.8 g) in MeOH (14 mL) was added dropwise to a solution of $HNMe_2$ (74 mmol, 3.34 g) in MeOH (20 mL). The mixture was stirred for 1.5 h at rt. Concentration of the reaction mixture gave a light yellow oil. Vacuum distillation afforded 7.30 g (90%) of a colorless oil (bp 103-104 °C/17 Torr).

^{1}H NMR (100 MHz): 1.13 (s, 3 H, C*H*$_3$-C), 1.60-1.90 (m, 2 H, C*H*$_2$CH$_2$CN), 2.31 (s, 2 H, C*H*$_2$-N), 2.38 (s, 6 H, (C*H*$_3$)$_2$N-), 2.40-2.60 (m, 2 H, C*H*$_2$CN). ^{13}C NMR: 11.5 (*C*2), 24.8 (*C*H$_3$-C), 35.6 (*C*3), 48.3 (*C*H$_3$-N), 68.4 (*C*5), 70.5 (*C*4), 120.5 (*C*N). IR: 3483 (OH), 2248 (C≡N), 1044 (N-C). MS *m/e* (relative

intensity): 156 (5.2), 141 (29.2), 102 (100), 87 (16.7), 59 54.2), 58 (27.6). Anal. Calcd for $C_8H_{16}N_2O$: C, 61.49; H, 10.34; N, 17.93. Found: C, 61.27; H, 10.49; N, 17.61.

(*RS*)-3-Cyano-1-[(dimethylamino)methyl]-1-methylpropyl 3,5-dinitrobenzoate (18). A solution of 3,5-dinitrobenzoyl chloride (23 mmol, 5.41 g) in CH_2Cl_2 (25 mL) was slowly added to a stirred mixture of **10** (21 mmol, 3.28 g,) and Et_3N (5 mmol, 0.50 g) in CH_2Cl_2 (30 mL) at 0 °C (ice-water bath). After the addition was complete, the reaction mixture was stirred for an additional 2 h at 0 °C. The pH was adjusted to 12 with 5% NaOH. The phases were separated and the aqueous solution was extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 solutions were washed with H_2O (2 × 20 mL), dried, and concentrated to give 8.05 g (100%) of a brown oil. ^1H NMR (100 MHz): 1.69 (s, 3 H, CH_3-C), 2.35 (s, 6 H, $(CH_3)_2N$), 2.48-2.60 (m, 4 H, CH_2CH_2CN), 2.79 (d, 1 H, *H*-CH-N, J_{app} = 7.2), 2.89 (d, 1 H, *H*-CH-N, J_{app} = 7.3), 9.11 (d, 2 H, *o-H* of benzoyl group, J_{app} = 0.9), 9.22 (t, 1 H, *p-H* of benzoyl group, J_{app} = 1.0). IR: 3104 (aromatic), 2249 (C≡N), 1728 (C=O), 1087 (C-N).

Resolution of (*R*)-18 and (*S*)-18. Racemic mixture of **18** was resolved by chiral column HPLC (CHIRALCEL OD column, 25 cm × 2 cm, J. T. Baker Inc., hexane:EtOH = 6:1, flowrate = 7 mL/min, injection size = 70 μg, retention time: 93 min, 105 min), giving (*R*)-**18** and (*S*)-**18**. Absolute configurations were not determined.

IR and ^1H NMR spectra are the same as **18**.

Aminolysis of 18. $HNMe_2$ (45 mmol, 2.03 g) was bubbled into a solution of **18** (21 mmol, 7.35 g) in MeOH (60 mL). The reaction mixture was stirred at rt for 0.5 h and then concentrated. The residue was taken up in H_2O (30 mL) and filtered to remove the light yellow solid *N,N*-dimethyl 3,5-dinitrobenzoate amide. The aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The extract was dried and concentrated to give a yellow oil. Vacuum distillation afforded 2.49 g (76%) of **10** as a colorless oil.

(*R*)-3-(1-Methyloxiranyl)-2-propenenitrile ((*R*)-20). A solution of oxalyl chloride (0.18 mol, 22.86 g) in CH_2Cl_2 (180 mL) was placed in a 1000 mL 3-neck round-bottom flask equipped with a thermometer, a $CaSO_4$ drying tube, and a dropping funnel and cooled to -50 to -60 °C (acetonitrile/acetone/CO_2). A solution of DMSO (0.39 mol, 30.42 g) in CH_2Cl_2 (60 mL) was added and stirred for 5 min, followed by the addition of (*R*)-**5** (0.124 mol, 11.25 g) in CH_2Cl_2 (75 mL). Stirring was continued for an additional 20 min. Et_3N (0.75 mol, 76 g) was added and the reaction mixture was stirred for 5 min and then allowed to warm to rt. H_2O (250 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 × 70 mL). The organic layers were combined, washed with satd brine (100 mL), 10% HCl (50 mL), H_2O (50 mL), 5% Na_2CO_3 (50 mL), and H_2O (50 mL), and dried, which was assumed to be a solution of (*S*)-(1-methyloxiranyl)formaldehyde ((*S*)-**19**) and CH_2Cl_2.

A solution of $(EtO)_2P(O)CH_2CN$ (0.135 mol, 24.03 g) in MeOH (50 mL) was added under N_2 to a stirred solution of CH_3OLi (0.135 mol) in MeOH

(prepared by adding 54 mL of 2.5 M solution of BuLi in hexanes into MeOH (50 mL) at 0 °C) maintained at 0 °C. Then the solution of (*S*)-**19** (above) was added to the mixture at 0 °C. The reaction mixture was stirred at rt overnight and then quenched with H_2O (50 mL). The organic phase was separated and washed with 10% HCl (50 mL) and H_2O (50 mL), dried and concentrated. The resulting oil was purified by column chromatography (Alumina, 80-200 mesh, hexane:ether = 1:10) to give 5.77 g (43% from (*R*)-**5**) of a colorless oil.

^{1}H NMR (100 MHz): 1.50 (s, 3 H, C*H*$_3$-), 2.76 (d, 1 H, C*H*=oxirane, J_{app} = 5.3), 2.92 (d, 1 H, C*H*=oxirane, J_{app} = 5.4), 5.59 (d, 1 H, C-C*H*=, J_{app} = 16.3), 6.60 (d, 1 H, =C*H*-CN, J_{app} = 16.4). IR: 3060 (olefinic C-H stretch), 2226 (CH=CH-C≡N), 1634 (C=C stretch), 908 (olefinic C-H bend).

(*R*) - 5 - (*N, N* - Dimethylamino) - 4 - hydroxy - 4 - methyl - 2 - pentenenitrile ((*R*)-21). A solution of (*R*)-**20** (52 mmol, 5.67 g) in MeOH (14 mL) was added dropwise to a solution of $HNMe_2$ (74 mmol, 3.34 g) in MeOH (20 mL). The reaction mixture was stirred for 1.5 h. Concentration of the reaction mixture gave a light yellow oil. Vacuum distillation afforded 7.10 g (89%) of a colorless oil.

^{1}H NMR (100 MHz): 1.22 (s, 3 H, C*H*$_3$-C), 2.30 (s, 6 H, (C*H*$_3$)$_2$N-), 2.45 (s, 2 H, -C*H*$_2$-), 5.78 (d, 1 H, C-C*H*=, J_{app} = 16.0), 6.74 (d, 1 H, =C*H*-CN, J_{app} = 15.8). IR: 3464 (OH), 3060 (olefinic C-H stretch), 2225 (CH=CH-C≡N), 1632 (C=C stretch), 1042 (N-C).

Compound (*R*)-10 (from (*R*)-21). A solution of (*R*)-**21** (5 mmol, 0.77 g) in absolute EtOH (20 mL) containing 10% Pd on charcoal (0.3 g) was hydrogenated at 25 psi (pressure reaction apparatus) and rt for 24 h. The reaction mixture was filtered and concentrated affording a colorless oil, which was purified by column chromatography (SiO_2, 70-230 mesh, methanol) (0.68 g, 87%).

IR and ^1H NMR spectra are the same as compound **10**.

(*S*)-(1-Methyloxiranyl)methyl 4-nitrobenzenesulfonate ((*S*)-22). A solution of 4-nitrobenzenesulfonyl chloride (19.4 mmol, 4.3 g) in CH_2Cl_2 (10 mL) was slowly added to a stirred mixture of (*R*)-2-methylglycidol (15.3 mmol, 1.39 g, 97% purity) and Et_3N (23.7 mmol, 2.37 g) in CH_2Cl_2 (10 mL) at 0 °C (ice-water bath). The reaction mixture was stirred for additional 3 h at 0 °C and then stored in freezer for 10-12 h. The reaction mixture was diluted with cold CH_2Cl_2 (10 mL) and filtered with suction to remove precipitated ammonium salt which then was washed with cold CH_2Cl_2 (10 mL). The mother liquor was washed with cold 5% HCl (10 mL), satd $NaHCO_3$ (10 mL), cold H_2O (2 × 8 mL), and dried. Concentration of the solution gave 4.06 g of a light yellow solid. Recrystallization from EtOH gave 3.26 g (78 %) of (*S*)-**22**.

^1H NMR (100 MHz): 1.37 (s, 3 H, CH_3-), 2.67 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7), 2.72 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7), 4.01 (d, 1 H, *H*-$CHOSO_2Ar$, J_{app} = 10.9), 4.27 (d, 1 H, *H*-$CHOSO_2Ar$, J_{app} = 10.9), 8.09-8.47 (m, 4 H, Bz).

(*R*)-2-Hydroxy-3-iodo-2-methylpropyl 4-nitrobenzenesulfonate ((*R*)-23). Compound (*S*)-**22** (0.18 mmol, 50 mg) was added to a solution of

NaI (0.3 mmol, 45 mg) in acetic acid (0.10 mL) and H_2O (0.01 mL) at 5 °C. After 1 h, the mixture was poured into H_2O (3 mL). The aqueous solution was extracted with Et_2O (3 × 2 mL). The combined Et_2O extracts were washed with satd $NaHCO_3$ until neutral (a trace of $NaHSO_3$ removed free I_2), then with H_2O (3 mL), and dried. Concentration of the solution gave a light yellow solid. Crystals for X-ray analysis were obtained by recrystallization from MeOH.

^1H NMR (100 Hz): 1.41 (s, 3 H, C*H*$_3$-), 3.31 (s, 2 H, IC*H*$_2$-), 4.11 (s, 2 H, C*H*$_2$-OS), 8.10-8.49 (m, 4 H, Bz).

***(2R, 6R; 2R, 6S; 2S, 6S; 2S, 6R)* - 2 - (2 - Cyanoethyl) - 6 - (methoxycarbonyl)methyl-2,4,4-trimethylmorpholinium bromide (7).** To a stirred solution of methyl-4-bromo-2-butenoate (14 mmol, 2.51 g) in CH_3NO_2 (30 mL) was added **10** (14 mmol, 2.18 g) dissolved in CH_3NO_2 (20 mL). The solution was stirred at 50-60 °C for 3 h. After cooling to rt, the reaction mixture was concentrated and the residue was washed with Et_2O. After drying under vacuum, it was dissolved in MeOH (130 mL) and refluxed for 18 h. Concentration of the reaction mixture gave a brown oil, which was washed with ether and dried under vacuum affording 4.70 g (100%) of a yellow oil, which was used for next reaction without further purification.

Methyl (*2S, 6R; 2R, 6S*) - 2 - [6 - (2 - cyanoethyl) - 4, 6 - dimethyl morpholinyl]acetate ((*2S,6R;2R,6S*)-8) and methyl (*2R,6R;2S,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate ((*2R, 6R; 2S, 6S*)-8). Crude **7** (14 mmol, 4.70 g) was dissolved in *N,N*-dimethylethanolamine (20

mL) at 50 °C under N_2. After 15 min, thiophenol (42 mmol, 4.6 g) was added in one portion, and the temperature was raised to 70 °C for 24 h. The reaction mixture was then cooled to rt. The pH was adjusted to 1-2 with 10% HCl. The mixture was extracted with hexanes (3 × 20 mL). To the aqueous layer, 10% NaOH was added until pH~12. The aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL). The combined CH_2Cl_2 extracts were washed with H_2O (2 × 20 mL) and dried. Concentration of the solution gave crude product **8** as a mixture of four stereoisomers. Separation of this mixture by column chromatography (SiO_2, 230-400 mesh, EtOAc:MeOH = 100:3) gave a solid ((*2R,6R;2S,6S*)-**8**, 0.36 g, mp 48.8-49.8 °C) and an oil ((*2S,6R; 2R,6S*)-**8**, 0.48 g) (total yield: 25% from **10**). Crystals of (*2R,6R;2S,6S*)-**8** for X-ray analysis were obtained by crystallization from hexanes.

(*2R,6R;2S,6S*)-**8**:

^{1}H NMR (400 MHz): 1.10 (s, 3 H, C*H*$_3$-C6), 1.50-1.58 (m, 1 H, *H*-CHCH$_2$CN), 1.67 (t, 1 H, *H*(ax)-C3, J_{app} = 10.8), 1.82 (d, 1 H, *H*(ax)-C5, J_{app} = 11.5), 2.21 (s, 3 H, C*H*$_3$-N), 2.32-2.52 (m, 4 H, C*H*$_2$CO$_2$CH$_3$, C*H*$_2$CN), 2.47 (d, 1 H, *H*(eq)-C5, J_{app} = 11.5), 2.68 (d, 1 H, *H*(eq)-C3, J_{app} = 10.9), 2.83 (m, 1 H, *H*-CHCH$_2$CN), 3.70 (s, 3 H, C*H*$_3$-O), 4.03 (m, 1 H, *H*-C2). ^{13}C NMR: 10.6 (*C*H$_2$CN), 24.6 (*C*H$_3$-C6), 29.6 (*C*H$_2$CH$_2$CN), 38.5 (*C*H$_2$COO), 46.0 (*C*H$_3$-N), 51.7 (*C*H$_3$-O), 59.1 (*C*3), 63.9 (*C*5), 66.3 (*C*2), 72.4 (*C*6), 120.6 (*C*N), 171.2 (*C*=O). IR: 2246 (C≡N), 1739 (C=O). MS *m/e* (relative intensity): 240 (11.6), 225 (0.5), 209 (24.0), 200 (1.1), 186 (4.9), 181 (1.1), 167 (9.1), 143 (42.7), 128 (20.8), 115 (7.0), 98 (24.1), 84 (98.2), 82 (9.4), 70 (46.7), 59 (19.9), 57 (21.1), 43

(100), 42 (68.2), 41 (29.2), 29 (6.5). Anal. Calcd for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 59.79; H, 8.26; N, 11.44.

(*2S,6R;2R,6S*)-**8**:

^{1}H NMR (400 MHz): 1.34 (s, 3 H, 6-CH_3), 1.64 (t, 1 H, *H*(ax)-C3, J_{app} = 10.8), 1.78 (m, 3 H, *H*(ax)-C5, CH_2CH$_2$CN), 2.22 (s, 3 H, CH_3-N), 2.32-2.48 (m, 5 H, *H*(eq)-C5, CH_2CO$_2$CH$_3$, CH_2CN), 2.75 (d, 1 H, *H*(eq)-C3, J_{app} = 10.9), 3.69 (s, 3 H, CH_3-O), 4.18 (m, 1 H, *H*-C2). ^{13}C NMR: 11.0 (*C*H$_2$CN), 20.3 (*C*H$_3$-C6), 36.7 (*C*H$_2$CH$_2$CN), 38.9 (*C*H$_2$COO), 46.3 (*C*H$_3$-N), 51.7 (*C*H$_3$-O), 59.9 (*C*3), 62.9 (*C*5), 66.6 (*C*2), 72.2 (*C*6), 120.4 (*C*N), 171.2 (*C*=O). IR: 2248 (C≡N), 1739 (C=O). MS *m/e* (relative intensity): 240 (10.4), 225 (0.7), 209 (23.1), 200 (1.1), 186 (3.6), 181 (1.1), 167 (5.5), 143 (36.9), 128 (17.9), 115 (6.0), 98 (25.0), 84 (97.5), 82 (8.6), 70 (37.5), 59 (17.9), 57 (17.6), 43 (100), 42 (59.2), 41 (26.6), 29 (5.6). Anal. Calcd for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 59.17; H, 8.73; N, 11.08.

Determination of optical purity of 2-methylglycidol by ^{1}H NMR. (*R*)-(+)-MTPA (2.05 g), $SOCl_2$ (4 mL) and NaCl (0.03 g) were refluxed for 50 h. The excess $SOCl_2$ was removed by vacuum evaporation and the residue was distilled to give (*R*)-(+)-MTPA-Cl.[82]

To a solution of 2-methylglycidol (0.15 mmol, 13.2 mg) and Et_3N (0.06 mL) in CH_2Cl_2 (1 mL) was added (*R*)-(+)-MTPA-Cl (0.10 mL). After being allowed to stand in refrigerator (0 °C) for 10 h, the reaction mixture was washed with 5% H_2SO_4 (2 mL), filtered, and concentrated affording the Mosher's ester.

^{1}H NMR (200 MHz):

(a) Mosher's ester of (*R*)-(+)-2-methylglycidol: 1.36 (s, 3 H, C-C*H*$_3$), 2.64 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.6), 2.77 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.6), 3.56 (s, 3 H, OC*H*$_3$), 4.23 (d, 1 H, *H*-CHO-MTPA, J_{app} = 11.9), 4.47 (d, 1 H, *H*-CHO-MTPA, J_{app} = 11.8), 7.35-7.68 (m, 5 H, aromatic).

(b) Mosher's ester of (*S*)-(-)-2-methylglycidol: 1.33 (s, 3 H, C-C*H*$_3$), 2.64 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7), 2.74 (d, 1 H, *H*-CH=oxirane, J_{app} = 4.7), 3.56 (s, 3 H, OC*H*$_3$), 4.16 (d, 1 H, *H*-CHO-MTPA, J_{app} = 11.8), 4.50 (d, 1 H, *H*-CHO-MTPA, J_{app} = 11.9), 7.35-7.68 (m, 5 H, aromatic).

The optical purities of (*R*)- and (*S*)-2-methylglycidols were determined by integration of the signals (dd) of -*H*$_2$CO-(+)-MTPA. The ee values are 88.4% for (*R*)-2-methylglycidol and 91.8% for (*S*)-2-methylglycidol.

Determination of optical purity of compound 13 by ^{1}H NMR. A solution of **13** (3 mmol, 0.711 g) in MeOH (9 mL) was added to a solution of HNMe$_2$ (12 mmol, 0.54 g) in MeOH (3 mL). The reaction mixture was stirred for 2 h and then concentrated. MeOH (3 mL) was added, and the precipitate was removed by filtration. The solution was concentrated. Again, MeOH (2 mL) was added, and the precipitate was removed by filtration. The solution was placed in freezer for 0.5 h. The liquid was pipetted out and concentrated by rotary evaporation and then placed under vacuum. Vacuum distillation of the residue gave 0.30 g (75%) of 3-dimethylamino-2-methylpropane-1,2-diol, **6**, as a colorless oil (bp: 150 °C/17 Torr). ^{1}H NMR (200 MHz): 1.07 (s, 3 H, C*H*$_3$-C), 2.37 (s, 6 H, C*H*$_3$-N), 2.43 (dd, 1 H, *H*-CHN, J_{app} = 13.7, 1.8), 2.57

(d, 1 H, *H*-CHN, J_{app} = 13.6), 3.46 (dd, 1 H, *H*-CHOH, J_{app} = 11.0, 1.7), 3.67 (d, 1 H, *H*-CHOH, J_{app} = 11.1). ^{13}C NMR: 23.7 (CH_3-C), 47.9 (CH_3-N), 67.9 (-CH_2-N), 70.7 (-CH_2-OH), 71.2 (*C*-OH). IR: 3405 (OH), 1044 (C-N). MS, FAB, m/e: 134 (M^+ + 1). Anal. Calcd for $C_6H_{15}NO_2$: C, 54.14; H, 11.28; N, 10.53. Found: C, 53.82; H, 11.10; N, 10.41.

To a solution of **6** (0.18 mmol, 24 mg) and Et_3N (0.05 mL) in CH_2Cl_2 (1 mL) was added (*R*)-(+)-MTPA-Cl (0.10 mL) at 0 °C. The reaction mixture was placed in a freezer for 10 h. NaOH solution (5%) was added until pH~12. The phases were separated and the aqueous solution was extracted with CH_2Cl_2 (3 × 2 mL). The combined CH_2Cl_2 extracts were washed with H_2O (2 × 5 mL), dried, and concentrated to give Mosher's ester, **15**, as an oil.

The optical purities of (*R*)- and (*S*)-**13** were determined by integration of the ^{1}H NMR signals of C*H*$_3$-C of Mosher's ester, **15**. For optical enriched **13**, the minor isomer was undetectable.

^{1}H NMR (400 MHz): 1.086 (s, C*H*$_3$-C) for Mosher's ester of (*S*)-**6**, 1.069 (s, C*H*$_3$-C) for Mosher's ester of (*R*)-**6**.

CHAPTER III

INHIBITION OF CAT BY FOUR STEREOISOMERS OF 6-CARBOXYLATOMETHYL-2-HYDROXYMETHYL-2,4,4-TRIMETHYL MORPHOLINIUM

The four stereoisomers of 6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium, **II**, were tested as specific inhibitors of CAT by Drs. Nóirín Nic a' Bháird and Rona R. Ramsay at UCSF. The results are presented and discussed in this chapter.

III.1. PREVIOUS WORK

Pigeon breast CAT indiscriminately binds both enantiomers of carnitine and acetylcarnitine,[103,104] which indicates a two-point recognition of carnitine, involving carboxylate and trimethylammonium groups. (*R*)-Carnitine and (*R*)-acetylcarnitine are substrates in the forward and reverse reactions respectively, while the *S* enantiomers are competitive inhibitors.[103,104] This stereospecificity indicates that the location of the CoA binding site must be closer to the hydroxyl on (*R*)-carnitine than the one on (*S*)-carnitine. (Figure III.1).[72]

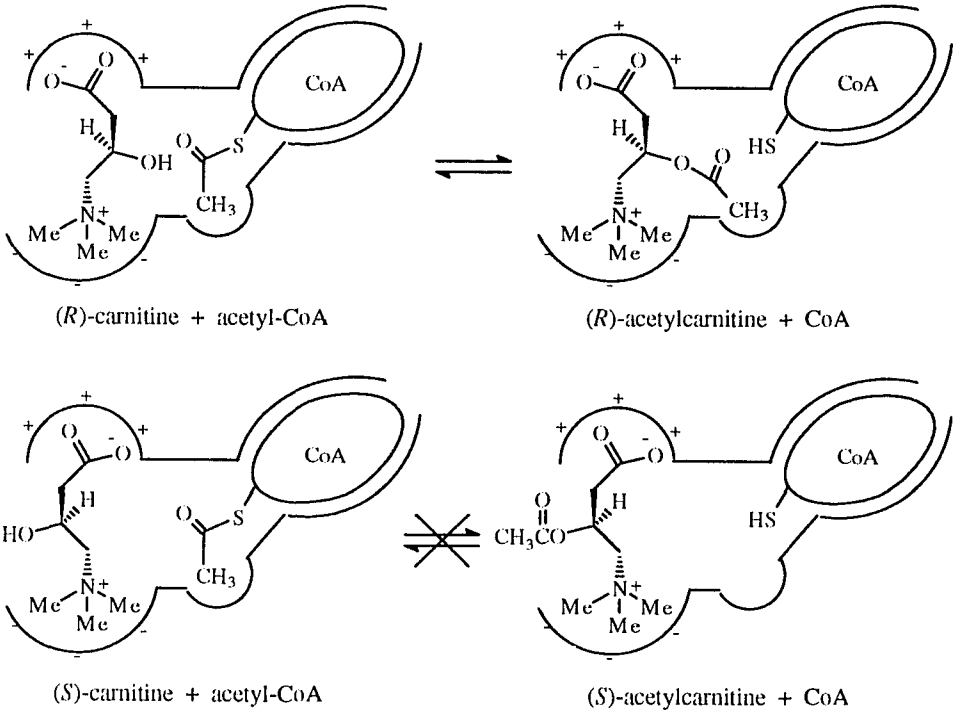
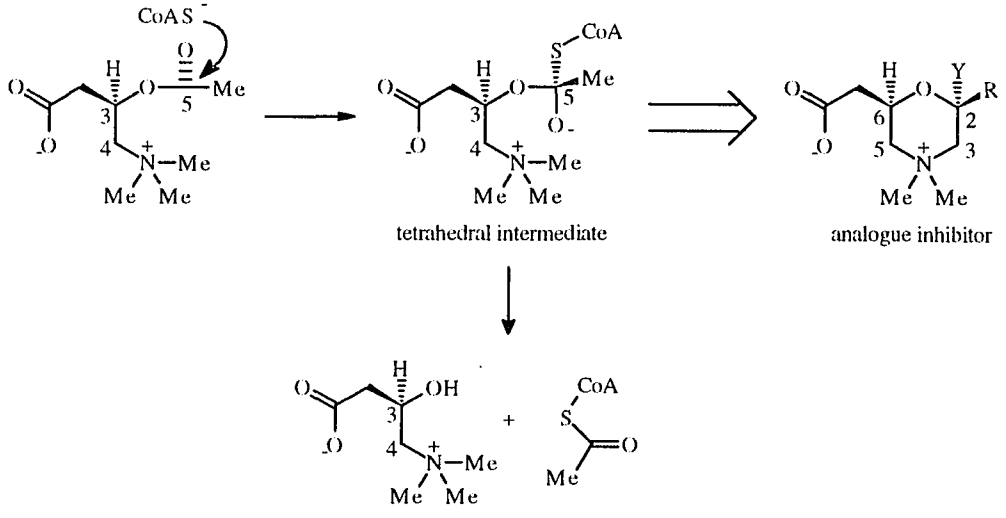
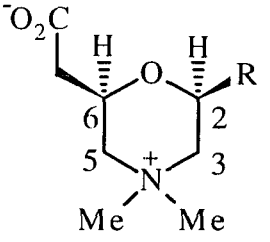


Figure III.1. Mode of substrate recognition in CAT



Scheme III.1

Based on the hypothesis for the mode of substrate recognition in CAT, a mechanism for the reversible acetyl transfer between carnitine and CoA has been proposed (Scheme III.1).[72] In this mechanism, the thiolate attacks on the *Re* face of the ester forming *R* configuration on the tetrahedral intermediate carbon.



	K_i (μM)
24. R = CH_2CO_2H	530
25. R = CH_3 (racemic)	1080
26. R = H (racemic)	1000

Figure III.2. Tetrahedral intermediate analogues and their K_i values with respect to (*R*)-carnitine

Three tetrahedral-intermediate analogues,[105] synthesized and assayed with CAT in 1987, (Figure III.2) must have the same configuration at C6 as (*R*)-carnitine does at C3 to effectively inhibit CAT. Of these analogues, **24** binds most strongly, with a K_i half that of the racemic compounds **25** and **26**. Because every molecule of **24** has one side that correctly matches the configuration of (*R*)-carnitine and only half of the molecules of racemic **25** and **26** have the correct configuration of (*R*)-carnitine, the twofold improvement in binding for **24** suggests that CAT is selectively binding one configuration of these inhibitors more tightly than the others (chiral recognition of C6). To confirm this speculation, more direct and potent

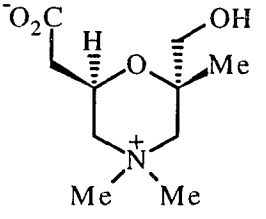
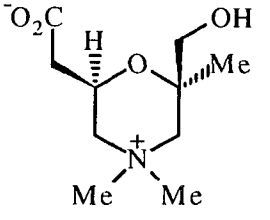
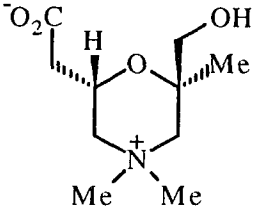
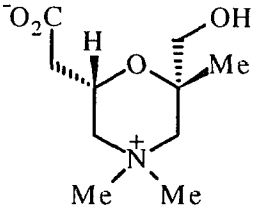
evidence is needed. In addition, those analogues do not test the configuration of the tetrahedral intermediate carbon (C2 in the analogues) because **24** is a meso (*RS*), **25** is a (*RS*;*SR*) racemate, and **26** is a (*R*;*S*) racemate. Inhibition studies with the four stereoisomers of **II** provide a test of the configuration stereocenters.

III.2. RESULTS AND DISCUSSION

The concentrations of the inhibitors needed for 50% inhibition (IC_{50}) of commercial pigeon breast CAT have been determined at 250 μM of (*R*)-acetylcarnitine (K_m = 350 μM). The data are shown in Table III.1. All the four stereoisomers of **II** inhibit CAT to varying degrees. Compound (2*S*,6*R*)-**II** is the most potent inhibitor of CAT among these four stereoisomers. The value of K_i for this stereoisomer shows that it binds 2-fold better than (*R*)-acetylcarnitine to CAT. The data in Table III.1 also indicate that CAT recognizes both the configurations of C6 and C2 in the tetrahedral-intermediate analogues.

Compound (2*S*,6*R*)-**II** binds 10-fold better than (2*S*,6*S*)-**II** to CAT. Both of them have the same configuration of C2. Compounds (2*R*,6*R*)-**II** binds 2.5-fold better than (2*R*,6*S*)-**II** to CAT. Both of them also have the same configuration of C2. The difference in both comparisons of inhibition of CAT is the difference of configurations of carnitine fragments (C6). These comparisons confirm the previous conclusion that CAT recognizes the configuration of C6 and strongly prefers *R* configuration.

Table III.1. Inhibition of 6-carboxylatomethyl-2-hydroxymethyl-2,4,4-trimethylmorpholinium on CAT

Inhibitor	Structure	IC_{50} (mM)	K_i (μM)
(*2S*, *6R*)-**II**		0.42	187
(*2R*, *6R*)-**II**		1.36	
(*2R*, *6S*)-**II**		3.41	
(*2S*, *6S*)-**II**		4.04	

Pigeon breast CAT binds enantiomers of both carnitine and acetylcarnitine equally well.[103,104] But only (*R*)-enantiomers can undergo acetyl transfer, which means that only (*R*)-enantiomers can form a tetrahedral-intermediate. When the tetrahedral-intermediate forms (acetyl-

CoA is attached to carnitine), the enzyme is probably in a different conformation than when it binds carnitine or acetylcarnitine. This enzymic conformation complements only the (*R*)-carnitine tetrahedral-intermediate. Therefore, the enzyme in this conformation should be inhibited by the reaction-intermediate analogues that have the same configuration as (*R*)-carnitine (C6). This speculation agrees with our results, confirming the hypothesis that the enzymic conformation for binding these analogues is different than the enzymic conformation that binds the substrates.

Compounds (*2S,6R*)-**II** and (*2R,6R*)-**II** have the same configuration on C6 but different configurations on C2. Compound (*2S,6R*)-**II** binds 3-fold better than (*2R,6R*)-**II** to CAT, suggesting that *S* configuration of C2 is required for enzyme recognition. This result strongly supports the mechanism for acetyl transfer between carnitine and CoA proposed by Gandour et al[72] (Scheme III.1). The idea is that the thiolate approaches the acyloxy from the less-congested side and attacks on the *Re* face of the ester to form *R* configuration on the tetrahedral-intermediate (C5). The *S* configuration in the analogue inhibitor (C2) is the same relative configuration as the *R* configuration for the tetrahedral-intermediate (C5). This idea agrees with the observation that (*2S,6R*)-**II** is the best inhibitor among four stereoisomers.

Compared with the previously synthesized analogue inhibitors **24**, **25**, and **26**, which are meso and racemic compounds and do not test the configuration of tetrahedral intermediate carbon (C2 in the analogues), our

analogues are chiral compounds so that they can test not only the configuration of carnitine fragment but also the configuration of tetrahedral intermediate carbon. Compounds (*2S,6R*)-**II**, **24**, and the more active enantiomers of **25** and **26** have the same configuration on C6. Racemic **25** and **26** have K_i values of 1080 and 1000 respectively. We assume that the more active enantiomers of **25** and **26** have K_i values of 540 and 500 respectively. Compound (*2S,6R*)-**II** binds nearly 3-fold better than **24** and the active enantiomers of **25** and **26** to CAT. The possible reasons for these results are that (1) the configuration of C2 in (*2S,6R*)-**II** fits the enzyme much better than the configurations of C2 in **24**, **25**, and **26** do, and (2) hydrogen bonding is important for (*2S,6R*)-**II**.

By comparing the IC_{50} values of (*2S,6R*)-**II** with (*2R,6R*)-**II** and (*2S,6R*)-**II** with (*2S,6S*)-**II**, we can see that the configuration of C6 is more crucial than that of C2. We speculate that C6 in the analogue mimics C3 in the tetrahedral-intermediate better than C2 in the analogue mimics C5 in the intermediate (Scheme III.1). Carbon 6 in the analogue and C3 in the intermediate have almost the same environment, while in the intermediate C5 is connected to CoA and a negatively charged oxygen and in the analogue C2 is in a six-membered ring and connected to a hydroxymethyl group. This difference might affect enzyme recognition. In addition, the binding of CoA to CAT is different from the interaction of hydroxymethyl with CAT. Therefore, the importance of the configuration of C5 in the tetrahedral-intermediate might be underestimated by this inhibitory study.

III.3. CONCLUSION

In conclusion, the results of the study verify firmly that the enzyme recognizes both configurations of C2 and C6 in the tetrahedral-intermediate analogues. If the enzyme binds these analogue inhibitors as it binds the tetrahedral-intermediate, we can conclude that the enzymic conformation for binding the substrates is different from the enzymic conformation that binds the tetrahedral-intermediate. CAT binds both *R* and *S* enantiomers of carnitine or acetylcarnitine but the chiral center of carnitine is not recognized. The chiral recognition of carnitine is caused by the binding of CoA. The thiolate of CoA attacks on the *Re* face of the ester of carnitine to form *R* configuration on the tetrahedral intermediate carbon. Only the tetrahedral-intermediate with *R* configurations on C3 and C5 forms in the acetyl transfer reaction between carnitine and CoA. Therefore, only (*R*)-enantiomers can undergo acetyl transfer.

CHAPTER IV

CONCLUSION

IV.1. RESULTS

We have developed new synthetic methods and added new members to the family of tetrahedral-reaction-intermediate analogue inhibitors. The inhibitory effects of these new analogues on CAT have been studied.

1. We have prepared four stereoisomers of 6-carboxylatomethyl-2-hydroxymethyl-2, 4, 4-trimethyl morpholinium, **II**, which are reaction-intermediate analogue inhibitors and will serve as precursors to four stereoisomers of 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethyl morpholinium, **III**, and the target molecules of the project. This is the first time that four stereoisomers of this type of acyltransferase inhibitors are made.

2. We have prepared methyl (*2S,6S;2R,6R*)- and (*2S,6R;2R,6S*)-2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**, which are precursors to molecules **III**.

3. We have resolved racemic mixture of 5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, which makes it possible that four

stereoisomers of 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethyl morpholinium, **III**, be made starting with nonchiral compound, 3-methyl-3-buten-1-ol.

4. The four stereoisomers of **II** have been tested as specific inhibitors of CAT. The results confirm the hypothesis for the mode of substrate recognition in CAT and the mechanism for acetyl transfer between carnitine and CoA.

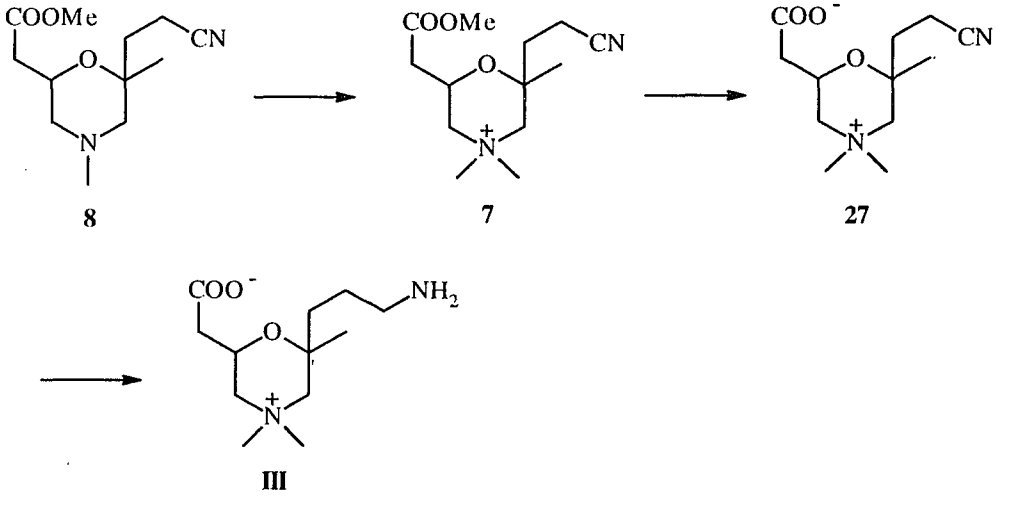
Although I did not get 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethylmorpholinium, **III**, due to various reasons, I came relatively close by preparing four stereoisomers of 6-carboxylatomethyl-2-hydroymethyl-2, 4, 4-trimethyl morpholinium, **II**, which are precursors to **III**, and resolving racemic mixture of 5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, which makes it possible that four stereoisomers of **III** be made starting with nonchiral compound, 3-methyl-3-buten-1-ol. Furthermore, compound **II** can lead to other compounds that might be potent inhibitors. (see below)

IV.2. FUTURE DIRECTION

The future work will be directed toward the synthesis of four stereoisomers of 2-(3-aminopropyl)-6-carboxylatomethyl-2,4,4-trimethyl morpholinium, **III**, which are precursors to the target molecules of the project.

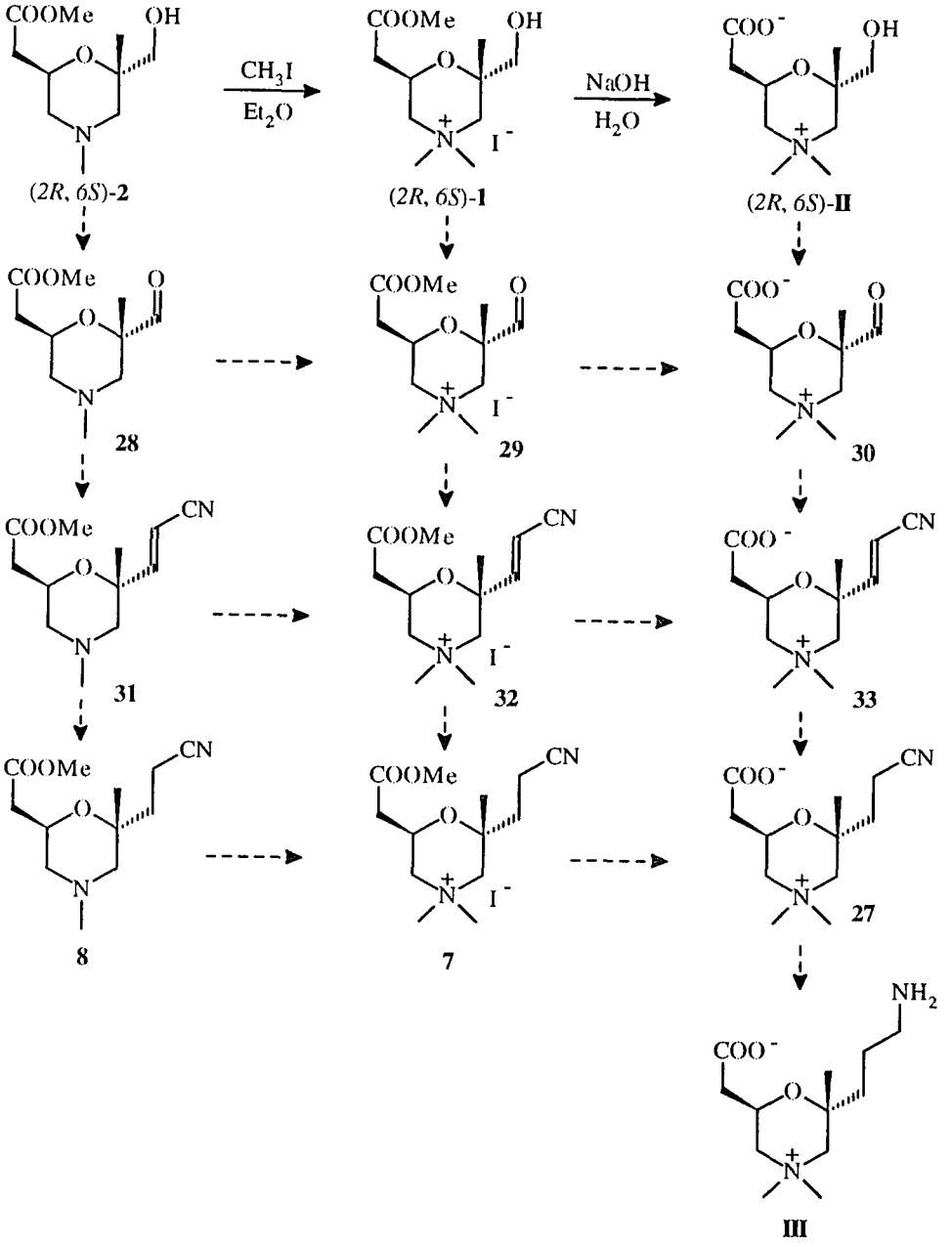
According to the results of our work (section IV.1), there are a number of possible synthetic routes for **III**, which are shown in Scheme IV.1 and Scheme IV.2.

Scheme IV.1 is a continuation of Scheme II.15. Methyl 2-[6-(2-cyanoethyl)-4,6-dimethylmorpholinyl]acetate, **8**, can be converted into compound **III** by methylation of tertiary amine, hydrolysis of methyl ester, and reduction of cyano group. The sequence of those three reactions might be crucial to this conversion. It is not difficult to find out the correct sequence. Four stereoisomers of **8** can be made from 3-methyl-3-buten-1-ol and optical resolution of (*RS*)-5-(*N,N*-dimethylamino)-4-hydroxy-4-methylpentanenitrile, **10**, is needed.



Scheme IV.1

In Scheme IV.2, compound **III** can be made in a number of ways. In these synthetic routes, three more analogue inhibitors, **27**, **30**, and **33**, can be made in addition to **III**. In synthetic route of Scheme IV.1, only one more analogue inhibitor, **27**, can be made in addition to **III**. Furthermore, compounds **1**, **2**, and **II** in Scheme IV.2 are made starting with chiral compound (*R*)- or (*S*)-2-methylglycidol (Scheme II.6), no optical resolution is needed. Therefore, Scheme IV.2 will be better than Scheme IV.1.


COOMe
OH
CH_3I
Et_2O
NaOH
H_2O
COO⁻
(2R, 6S)-2
(2R, 6S)-1
(2R, 6S)-II
28
29
30
31
32
33
8
7
27
CN
NH_2
III

Scheme IV.2

BIBLIOGRAPHY

1. Rebouche, C. J. "Carnitine Metabolism and Function in Humans." *Ann. Rev. Nutri.* **1986**, *6*, 41-66.

2. Bieber, L. L. "Carnitine." *Ann. Rev. Biochem.* **1988**, *57*, 261-283.

3. Schulz, H. "Beta Oxidation of Fatty Acids." *Biochim. Biophys. Acta* **1991**, *1081*, 109-120.

4. Coates, P. M.; Tanaka, K. "Molecular Basis of Mitochondrial Fatty Acid Oxidation Defects." *J. Lipid Res.* **1992**, *33*, 1099-1110.

5. Borum, P. R. "Carnitine." *Ann. Rev. Nutri.* **1983**, *3*, 233-259.

6. Sekas, G.; Paul, H. S. "Inhibition of Carnitine Acyltransferase Activities by Bile Acids in Rat Liver Peroxisomes." *Biochim. Biophys. Acta* **1992**, *1125*, 196-202.

7. Paul, H. S.; Sekas, G.; Adibi, S. A. "Carnitine Biosynthesis in Hepatic Peroxisomes." *Eur. J. Biochem.* **1992**, *203*, 599-605.

8. Cress, A. P.; Fraker, P. J.; Bieber, L. L. "Carnitine and Acylcarnitine Levels of Human Peripheral Blood Lymphocytes and Mononuclear Phagocytes." *Biochim. Biophys. Acta* **1989**, *992*, 135-139.

9. Anonymous. "A Role for Carnitine in Medium-Chain Fatty Acid Metabolism?" *Nutri. Rev.* **1991**, *49*, 243-245.

10. Ramsay, R. R.; Arduini, A. "Perspective in Biochemistry and Biophysics. The Carnitine Acyltransferases and Their Role in Modulating Acyl-CoA Pools." *Arch. Biochem. Biophys.* **1993**, *302*, 307-314.

11. Engel, A. G.; Angelini, C. "Carnitine Deficiency of Human Skeletal Muscle with Associated Lipid Storage Myopathy. New Syndrome." *Science* **1973**, *179*, 899-902.

12. Sandor, A. "Butyrobetaine Is Equal to L-Carnitine in Elevating L-Carnitine Levels in Rats." *Biochim. Biophys. Acta* **1991**, *1083*, 135-138.

13. Bowman, B. A. B. "Acetyl-Carnitine and Alzheimer's Diease." *Nutri. Rev.* **1992**, *50*, 142-144.

14. Feller, A. G.; Rudman, D. "Role of Carnitine in Human Nutrition." *J. Nutri.* **1988**, *118*, 541-547.

15. Brady, P. S.; Ramsay, R. R.; Brady, L. J. "Regulation of the Long-Chain Carnitine Acyltransferases." *FASEB J.* **1993**, *7*, 1039-1044.

16. Colucci, W. J.; Gandour, R. D. "Carnitine Acetyltransferase: A Review of Its Biology, Enzymology, and Bioorganic Chemistry." *Bioorg. Chem.* **1988**, *16*, 307-334.

17. Miyazawa, S.; Ozasa, H; Osumi, T.; Hashimoto, T. "Purification and Properties of Carnitine Octanoyltransferase and Carnitine Palmitoyltransferase from Rat Liver." *J. Biochem.* **1983**, *94*, 529-542.

18. Ramsay, R. R.; Derrick, J. P.; Friend, A. S.; Tubbs, P. K. "Purification and Properties of the Soluble Carnitine Palmitoyltransferase from Bovine Liver Mitochondria." *Biochem. J.* **1987**, *244*, 271-278.

19. Chatterjee, B.; Song, C. S.; Kim, J. M.; Ray, A. K. "Cloning, Sequencing, and Regulation of Rat Liver Carnitine Octanoyltransferase: Transcriptional Stimulation of the Enzyme during Peroxisome Proliferation." *Biochemistry* **1988**, *27*, 9000-9006.

20. Farrell, S. O.; Fiol, C. J.; Reddy, J. K.; Bieber, L. L. "Properties of Purified Carnitine Acyltransferases of Mouse Liver Peroxisomes." *J. Biol. Chem.* **1984**, *259*, 13089-13095.

21. Chung, C.-D.; Bieber, L. L. "Properties of the Medium Chain/Long Chain Carnitine Acyltransferase Purified from Rat Liver Microsomes." *J. Biol. Chem.* **1993**, *268*, 4519-4524.

22. Declercq, P. E.; Falck, J. R.; Kuwajima, M.; Tyminski, H.; Foster, D. W.; McGarry, J. D. "Characterization of the Mitochondrial Carnitine Palmitoyltransferase Enzyme System. I. Use of Inhibitors." *J. Biol. Chem.* **1987**, *262*, 9812-9821.

23. Murthy, M. S. R.; Pande, S. V. "Characterization of a Solubilized Malonyl-CoA-sensitive Carnitine Palmitoyltransferase from the Mitochondrial Outer Membrane as a Protein Distinct from the

Malonyl-CoA-Insensitive Carnitine Palmitoyltransferase of the Inner Membrane." *Biochem. J.* **1990**, *268*, 599-604.

24. Kolodziej, M. P.; Crilly, P. J.; Corstorphine, C. G.; Zammit, V. A. "Development and Charaterization of a Polyclonal Antibody against Rat Liver Mitochondrial Overt Carnitine Palmitoyltransferase (CPT I)." *Biochem. J.* **1992**, *282*, 415-421.

25. Bergstrom, J. D.; Reitz, R. C. "Studies on Carnitine Palmitoyl Transferase: the Similar Nature of CPT_I (Inner Form) and CPT_O (Outer Form)." *Arch. Biochem. Biophys.* **1980**, *204*, 71-79.

26. Clarke, P. R. H.; Bieber, L. L. "Isolation and Purification of Mitochondrial Carnitine Octanoyltransferase Activities from Beef Heart." *J. Biol. Chem.* **1981**, *256*, 9861-9868.

27. Woeltje, K. F.; Esser, V.; Weis, B. C.; Sen, A.; Cox, W. F.; McPhaul, M. J.; Slaughter, C. A.; Foster, D. W.; McGarry, J. D. "Cloning, Sequencing, and Expression of cDNA Encoding Rat Liver Mitochondrrial Carnitine Palmitoyltransferase II." *J. Biol. Chem.* **1990**, *265*, 10720-10725.

28. Lilly, K.; Bugaisky, G. E.; Umeda, P. K.; Bieber, L. L. "The Medium-Chain Carnitine Acyltransferase Activity Associated with Rat Liver Microsomes Is Malony-CoA Sensitive." *Arch. Biochem. Biophys.* **1990**, *280*, 167-174.

29. Derrick, J. P.; Ramsay, R. R. "L-Carnitine Acyltransferase in Intact Peroxisomes Is Inhibited by Malonyl-CoA." *Biochem. J.* **1989**, *262*, 801-806.

30. Ishii, S.; Ishii, H.; Watanabe, T.; Suga, T. "Purification and Properties of Peroxisomal Carnitine Palmitoyltransferase in Chick Embryo Liver." *Biochim. Biophys. Acta* **1987**, *924*, 530-542.

31. Ramsay, R. R.; Mancinelli, G.; Arduini, A. "Carnitine Palmitoyltransferase in Human Erythrocyte Membrane." *Biochem. J.* **1991**, *275*, 685-688.

32. McMillin, J. B.; Hudson, E. K.; Van Winkle, W. B. "Evidence for Malonyl-CoA-Sensitive Carnitine Acyl-CoA Transferase Activity in Sarcoplasmic Reticulum of Canine Heart." *J. Mol. Cell. Cardiol.* **1992**, *24*, 259-268.

33. Marquis, N. R.; Fritz, I. B. "The Distribution of Carnitine, Acetylcarnitine, and Carnitine Acetyltransferase in Rat Tissues." *J. Biol. Chem.* **1965**, *240*, 2193-2196.

34. Fritz, I. B. "Carnitine and Its Role in Fatty Acid Metabolism." *Advances in Lipid Research*; Paoletti, R., Kritchevsky, D., Eds.; Academic Press: New York, **1963**; Vol. 1, pp 285-334.

35. McCaman, R. E.; McCaman, M. W.; Stafford, M. L. "Carnitine Acetyltransferase in Nervous Tissue." *J. Biol. Chem.* **1966**, *241*, 930-934.

36. Chase, J. F. A.; Pearson, D. J.; Tubbs, P. K. "The Preparation of Crystalline Carnitine Acetyltransferase." *Biochim. Biophys. Acta* **1965**, *96*, 162-165.

37. Edwards, Y. H.; Chase, J. F. A.; Edwards, M. R.; Tubbs, P. K. "Carnitine Acetyltransferase: The Question of Multiple Forms." *Eur. J. Biochem.* **1974**, *46*, 209-215.

38. Chase, J. F. A. "Carnitine Acetyltransferase from Pigeon Breast Muscle." *Methods in Enzymology*; Lowenstein, J. M., Ed.; Academic Press: New York, **1970**; Vol. 13, p 387.

39. Chase, J. F. A. "pH-Dependence of Carnitine Acetyltransferase Activity." *Biochem. J.* **1967**, *104*, 503-509.

40. Fritz. I. B., Schultz, S. K.; Srere, P. A. "Properties of Partially Purified Carnitine Acetyltransferase." *J. Biol. Chem.* **1963**, *238*, 2509-2517.

41. Ueda, M.; Tanaka, A.; Fukui, S. "Peroxisomal and Mitochondrial Carnitine Acetyltransferases in Alkane-Grown Yeast *Candida tropicalis*." *Eur. J. Biochem.* **1982**, *124*, 205-210.

42. Claus, R.; Käppeli, O.; Fiechter, A. "Purification of Carnitine Acetyltransferase from *Candida tropicalis*." *Anal. Biochem.* **1982**, *127*, 376-379.

43.. Kispal, G.; Cseko, J.; Alkonyi, I.; Sandor, A. "Isolation and Characterization of Carnitine Acetyltransferase from *S. cerevisiae*." *Biochim. Biophys. Acta* **1991**, *1085*, 217-222.

44. Mittal, B.; Kurup, C. K. R. "Purification of Clofibrate-Induced Carnitine Acetyltransferase from Rat Liver Mitochondria." *Biochim. Biophys. Acta* **1980**, *619*, 90-97.

45. Miyazawa, S.; Ozasa, H.; Furuta, S.; Osumi, T.; Hashimoto, T. "Purification and Properties of Carnitine Acetyltransferase from Rat Liver." *J. Biochem.* **1983**, *93*, 439-451.

46. Markwell, M. A. K.; Tolbert, N. E.; Bieber, L. L. "Comparison of the Carnitine Acyltransferase activities from Rat Liver Peroxisomes and Microsomes." *Arch. Biochem. Biophys.* **1976**, *176*, 479-488.

47. Huckle, W. R.; Tamblyn, T. M. "Purification and Properties of Carnitine Acetyltransferases from Bovine Spermatozoa and Heart." *Arch. Biochem. Biophys.* **1983**, *226*, 94-110.

48. Bloisi, W.; Colombo, I.; Garavaglia, B.; Giardini, R.; Finocchiaro, G.; Didonato, S. "Purification and Properties of Carnitine Acetyltransferase from Human Liver." *Eur. J. Biochem.* **1990**, *189*, 539-546.

49. Harris, R. C.; Foster, C. V. L.; Hultman, E. "Acetylcarnitine Formation during Intense Muscular Contraction in Humans." *J. Appl. Physiol.* **1987**, *63*, 440-442.

50. Cederblad, G.; Carlin, J. I.; Constantin-Teodosiu, D.; Harper, P.; Hultman, E. "Radioisotopic Assays of CoASH and Carnitine and Their Acetylated Forms in Human Skeletal Muscle." *Anal. Biochem.* **1990**, *185*, 274-278.

51. Chalmers, R. A.; Roe, C. R.; Tracey, B. M.; Stacey, T. E.; Hoppel, C. L.; Millington, D. S. "Secondary Carnitine Insufficiency in Disorders of Organic Acid Metabolism: Modulation of Acyl-CoA/CoA Ratios by L-Carnitine *in vivo*." *Biochem. Soc. Trans.* **1983**, *11*, 724-725.

52. Brass, E. P.; Fennessey, P. V.; Miller, L. V. "Inhibition of Oxidative Metabolism by Propionic Acid and Its Reversal by Carnitine in Isolated Rat Hepatocytes." *Biochem. J.* **1986**, *236*, 131-577.

53. Brass, E. P.; Ruff, L. J. "Effect of Carnitine on Propionate Metabolism in the Vitamin B-12-deficient Rat." *J. Nutr.* **1989**, *119*, 1196-1202.

54. Brass, E. P.; Beyerinck, R. A. "Interactions of Propionate and Carnitine Metabolism in Isolated Rat Hepatocytes." *Metabolism* **1987**, *36*, 781-787.

55. Idell-Wenger, J. A.; Grotyohann, L. W.; Neely, J. R. "Regulation of Fatty Acid Utilization in Heart. Role of the Carnitine-Acetyl-CoA Transferase and Carnitine-Acetylcarnitine Translocase System." *J. Mol. Cell. Cardiol.* **1982**, *14*, 413-417.

56. Beenakkers, A. M. T.; Klingenberg, M. "Carnitine-Coenzyme A Transacetylase in Mitochondria from Various Organs." *Biochim. Biophys. Acta* **1964**, *84*, 205-207.

57. Pearson, D. J.; Tubbs, P. K. "Carnitine and Derivatives in Rat Tissues." *Biochem. J.* 1967, *105*, 953-963.

58. DiDonato, S.; Rimoldi, M.; Moise, A.; Bertagnolio, B.; Uziel, G. "Fatal Ataxic Encephalopathy and Carnitine Acetyltransferase Deficiency: A Functional Defect of Pyruvate Oxidation?" *Neurology* **1979**, *29*, 1578-1583.

59. Przyrembel, H. "Therapy of Mitochondrial Disorders." *J. Inherited Metab. Dis.* **1987**, *10*, 129-146.

60. Kalaria, R. N.; Harik, S. I. "Carnitine Acetyltransferase Activity in the Human Brain and its Microvessels Is Decreased in Alzheimer's Disease." *Ann. Neurol.* **1992**, *32*, 583-586.

61. Kispal, G.; Sumegi, B.; Dietmeier, K.; Bock, I.; Gajdos, G.; Tomcsanyi, T.; Sandor, A. "Cloning and Sequencing of a cDNA Encoding *Saccharomyces cerevisiae* Carnitine Acetyltransferase." *J. Biol. Chem.* **1993**, *268*, 1824-1829.

62. Schmalix, W.; Bandlow, W. "The Ethanol-inducible *YAT1* Gene from Yeast Encodes a Presumptive Mitochondrial Outer Carnitine Acetyltransferase." *J. Biol. Chem.* **1993**, *268*, 27428-27439.

63. Vessey, D. A.; Chen, W.; Ramsay, R. R. "Effect of Carboxylic Acid Xenobiotics and Their Metabolites on the Activity of Carnitine Acyltransferases." *Biochem. J.* **1991**, *279*, 895-897.

64. Lilly, K.; Chung, C.; Kerner, J.; VanRenterghem, R.; Bieber, L. L. "Effect of Etomoxiryl-CoA on Different Carnitine Acyltransferases." *Biochem. Pharm.* **1992**, *43*, 353-361.

65. Saeed, A.; McMillin, J. B.; Wolkowicz, P. E.; Brouillette, W. J. "Carnitine Acyltransferase Enzymic Catalysis Requires a Positive Charge on the Carnitine Cofactor." *Arch. Biochem. Biophys.* **1993**, *305*, 307-312.

66. Brouillette, W. J.; Saeed, A.; McMillin, J. B.; Wolkowicz, P. E. "5,5-Dimethyl-3-Aminohexanoic Acid: Synthesis, Resolution, and Studies with Carnitine Acyltransferases." *Poster Presentation, Division of Medicinal Chemistry, Abstract 251*, 207th National ACS Meeting, San Diego, CA, March 13-17,1994.

67. Brouillette, W. J; Saeed, A.; McMillin, J. B.; Wolkowicz, P. E.; Abuelyaman, A.; Hutchison, T. "Rigid Carnitine Analogs: Synthesis, Resolution, and Studies with Carnitine Acyltransferases." *Poster Presentation, Division of Medicinal Chemistry, Abstract 252*, 207th National ACS Meeting, San Diego, CA, March 13-17,1994.

68. Sekas, G.; Paul, H. S. "Inhibition of Carnitine Acetyltransferase by Bile Acids: Implications for Carnitine Analysis." *Anal. Biochem.* **1989**, *179*, 262-267.

69. Sekas, G.; Paul, H. S. "Inhibition of Carnitine Acyltransferase Activities by Bile Acids in Rat Liver Peroxisomes." *Biochim. Biophys. Acta* **1992**, *1125*, 196-202.

70. Gandour, R. D.; Blackwell, N. L.; Colucci, W. J.; Chung, C.; Bieber, L. L.; Ramsay, R. R.; Brass, E. P.; Fronczek, F. R. "Syntheses, Structures, and Enzymatic Evaluations of Hemiacylcarnitiniums, A New Class of Carnitine Acyltransferase Inhibitors." *J. Org. Chem.* **1992**, *57*, 3426-3431.

71. Gandour, R. D.; Colucci, W. J.; Stelly, T. C.; Brady, P. S.; Brady, L. J. "Active-Site Probes of Carnitine Acyltransferases. Inhibition of Carnitine Acetyltransferase by Hemiacetylcarnitinium, A Reaction Intermediate Analogue." *Biochem. Biophys. Res. Commun.* **1986**, *138*, 735-741.

72. Gandour, R. D.; Colucci, W. J.; Fronczek, F. R. "Crystal Structures of Carnitine and Acetylcarnitine Zwitterions: A Structural Hypothesis for Mode of Action." *Bioorg. Chem.* **1985**, *13*, 197-208.

73. Brady, L. J.; Brady, P. S.; Gandour, R. D. "Effect of Methylglyoxal bis(Guanylhydrazone) on Hepatic, Heart and Skeletal Muscle Mitochondrial Carnitine Palmitoyltransferase and β-Oxidation of Fatty Acids." *Biochem. Pharm.* **1987**, *36*, 447-452.

74. Moody, D. E.; Narloch, B. A.; Shull, L. R.; Hammock, B. D. "The Effect of Structurally Divergent Herbicides on Mouse Liver Xenobiotic-Metabolizing Enzymes (P-450-Dependent Monooxygenases, Epoxide Hydrolases and Glutathione *S*-transferases) and Carnitine Acetyltransferase." *Toxicology Letters* **1991**, *59*,175-185.

75. Fritz, I. B.; Shultz, S. K. "Carnitine Acetyltransferase. II. Inhibition by Carnitine Analogues and by Sulfhydryl Reagents." *J. Biol. Chem.*, **1965**, *240*, 2188-2192.

76. Colucci, W. J.; Gandour, R. D.; Mooberry, E. S. "Conformational Analysis of Charged Flexible Molecules in Water by Application of a New Karplus Equation Combined with MM2 Computation: Conformations of Carnitine and Acetylcarnitine." *J. Am. Chem. Soc.*, **1986**, *108*, 7141-7147.

77. Wolfenden, R. "Transition-State Affinity as a Basis for the Design of Enzyme Inhibitors." *Transition States of Biochemical Processes*; Gandour, R.D.; Schowen, R. L. Eds.; Plenum Press: New York and London, **1978**; Chap. 15, pp 555-578.

78. Hupe, D. J.; Jencks, W. P. "Nonlinear Structure-Reactivity Correlations. Acyl Transfer between Sulfur and Oxygen Nucleophiles." *J. Am. Chem. Soc.*, **1977**, *99*, 451-464.

79. Nic a' Bháird, N.; Kumaravel, G.; Gandour, R. D.; Krueger, M. J.; Ramsay, R. R. "Comparison of the Active Sites of the Purified Carnitine Acyltransferases from Peroxisomes and Mitochondria by using a Reaction-Intermediate Analogue." *Biochem. J.*, **1993**, *294*, 645-651.

80. Asknes, G.; Prue, J. E. "Kinetic Salt Effects and Mechanism in the Hydrolysis of Positively Charged Esters." *J. Chem. Soc.*, **1959**, 103-107.

81. Gandour, R. D.; Leung, O.-t.; Greway, A. T.; Ramsay, R. R.; Nic a' Bháird, N.; Fronczek, F. R.; Bellard, B. M.; Kumaravel, G. "(+)-Hemipalmitoylcarnitinium Strongly Inhibits Carnitine Palmitoyltransferase-I in Intact Mitochondria." *J. Med. Chem.*, **1993**, *36*, 237-242.

82. Dale, J. A.; Dull, D. L.; Mosher, H. S. "α-Methoxy-α-Trifluoromethylphenylacetic Acid, A Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines." *J. Org. Chem.* **1969**, *34*, 2543-2549.

83. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. "Catalytic Asymmetric Epoxidation and Kinetic Resolution: Modified Procedures Including in Situ Derivatization." *J. Am. Chem. Soc.* **1987**, *109*, 5765-5780.

84. Shing, T. K. M.; Tsui, H.-C.; Zhou, Z.-H. "First Total Synthesis of Potent Antitumor Agent (+)-Goniopypyrone." *Tetrahedron Lett.* **1993**, *34*, 691-692.

85. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry, 3rd Edt, Part A: Structure and Mechanisms*; Plenum Press: New York. **1990**; pp 163-166.

86. Broubham, P.; Cooper, M. S.; Cummerson, D. A.; Heaney, H.; Thompson, N. "Oxidation Reactions Using Magnesium Monoperphthalate: A Comparison with *m*-Chloroperoxybenzoic Acid." *Synthesis* **1987**, 1015-1017.

87. Cohen, J. F. "Epoxynitrile Cyclizations. Total Synthesis of *dl*-Grandisol." *Ph.D. Dissertation*, Chemistry Department, Columbia University, **1973**.

88. Grob, C. A.; Waldner, A. "Solvolysis of 4-Substituted 2-Chloro-2-Methylbutanes." *Helv. Chim. Acta* **1979**, *62*, 1854-1865.

89. Maillard, B.; Kharrat, A.; Rakotomanana, F.; Montaudon, E. "Intramolecular Homolytic Displacements 7—Free Radical Additions

to Unsaturated Peroxy Compounds: Synthesis of Oxygenated Heterocycles." *Tetrahedron* **1985**, *41*, 4047-4056.

90. Cornforth, J. W.; Cornforth, R. H.; Mathew, K. K. "A General Stereoselective Synthesis of Olefins." *J. Chem. Soc.* **1959**, 112-127.

91. Klunder, J. M.; Onami, T.; Sharpless, K. B. "Arenesulfonate Derivatives of Homochiral Glycidol: Versatile Chiral Building Blocks for Organic Synthesis." *J. Org. Chem.* **1989**, *54*, 1295-1304.

92. Johnson, C. R.; Dutra, G. A. "Reaction of Lithium Diorganocuprates(I) with Tosylates. I. Synthetic Aspects." *J. Am. Chem. Soc.* **1973**, *95*, 7777-7782.

93. Sun, G.; Fronczek, F. R.; Gandour, R. D. "(*R*)-(1-Methyloxiranyl)methyl 4-Nitrobenzenesulfonate." *Acta Crystallogr., Sect. C*, **1992**, *48*, 758-760.

94. Sun, G.; Fronczek, F. R.; Gandour, R. D. "(*R*)-2-Hydroxy-3-Iodo-2-Methylpropyl 4-Nitrobenzenesulfonate." *Acta Crystallogr., Sect. C*, **1993**, *49*, 1507-1509.

95. Gajewski, J. J; Gilbert, K. E. *PCMODEL Molecular Modeling Package, version 4.0.* **1992**. Serena Software, PO Box 3076, Bloomington, IN 47402, USA.

96. Wheland, G. W. *Advanced Organic Chemistry, 3rd Edt*; John Wiley: New York. **1960**; p 660.

97. Colucci, W. J. "Conformational Studies of Carnitine and Acetylcarnitine: Design and Synthesis of Active-Site Probes of Carnitine Acetyltransferase." *Dissertation*, Chemistry Department, Louisiana State University, **1987**.

98. Vaughan, W. R.; Baumann, J. B. "Reaction of Alkyl Carboxylic Esters with Mercaptides." *J. Org. Chem.* **1962**, *27*, 739-744.

99. Sheehan, J. C.; Daves, Jr. G. D. "Facile Alkyl-Oxygen Ester Cleavage." *J. Org. Chem.* **1964**, *29*, 2006-2008.

100. Sun, G.; Fronczek, F. R.; Gandour, R. D. "Methyl (2S,6S;2R,6R)-2-[6-(2-Cyanoethyl)-4,6-Dimethylmorpholinyl]acetate." *Acta Crystallogr., Sect. C*, **1994**, *50*, in press.

101. Serena Software. *GMMX* (*GLOBAL-MMX, version 1.0*), *Steric Energy Minimization Program*. **1992**. Serena Software, PO Box 3076, Bloomington, IN 47402, USA.

102. Paquette, L. A.; Sauer, D. R.; Cleary, D. G.; Kinsella, M. A.; Blackwell, C. M.; Anderson, L. G. "Application of Palladium-catalyzed [3 + 2] Cycloaddition Technology to the Elaboration of Kempane Diterpenes. Stereocontrolled Synthesis of (±)-3α-Hydroxy-7β-kemp-8(9)-en-6-one and (±)-3β-Hydroxykemp-7(8)-en-6-one." *J. Am. Chem. Soc.* **1992**, *114*, 7375-7387.

103. Chase, J. F. A.; Tubbs, P. K. "Some Kinetic Studies on Mechanism of Action of Carnitine Acetyltransferase." *Biochem. J.* **1966**, *99*,32-40.

104. Tipton, K. F.; Chase, J. F. A. "The Effects of Substrates on the Optical Rotatory Dispersion of Carnitine Acetyltransferase." *Biochem. J.* **1969**, *115*, 517-521.

105. Colucci, W. J.; Gandour, R. D.; Fronczek, F. R.; Brady, P. S.; Brady, L. J. "A "Siameso" Inhibitor: Chiral Recognition of a Prochiral Bilaterally Symmetric Molecule by Carnitine Acetyltransferase." *J. Am. Chem. Soc.* **1987**, *109*, 7915-7916.

APPENDIX A

CRYSTALLOGRAPHIC DATA FOR (2*S*,6*R*)-2-HYDROXYMETHYL-6-(METHOXYCARBONYL)METHYL-2,4,4-TRIMETHYLMORPHOLINIUM IODIDE

$C_{11}H_{22}NO_4I$, Mr = 359.2, orthorhombic, $P2_12_12_1$, a = 9.5519(6), b = 12.4000(6), c = 25.723(2) Å, V = 3046.7(6) Å^3, Z = 8, D_x = 1.564 g cm^{-3} at 293 K, λ (Mo $K\alpha$) = 0.71073 Å, μ = 20.8 cm^{-1}, $F(000)$ = 1440, 6251 unique data measured, final R = 0.027 for 5230 reflections with I > 3σ(I). Two independent formula units in asymmetric unit.

Table A.1. Coordinates and isotropic thermal parameters

$$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}\, a_i^* a_j^*\, \mathbf{a}_i \mathbf{a}_j$$

Atom	x	y	z	B_{eq} (Å^2)
I1	0.23568(3)	0.21429(2)	0.59731(1)	3.927(5)
I2	0.68574(3)	0.21211(2)	0.08180(1)	4.820(6)
O1A	0.2750(3)	0.2618(2)	0.17058(9)	3.03(5)
O2A	0.4204(3)	-0.0606(2)	0.2416(1)	5.49(8)
O3A	0.1908(3)	-0.0369(2)	0.2450(1)	5.71(8)
O4A	-0.0145(3)	0.3600(2)	0.0912(1)	4.07(6)
N1A	0.2419(3)	0.1345(2)	0.0766(1)	2.73(5)
C1A	0.3112(4)	0.0927(3)	0.1253(1)	2.91(7)
C2A	0.2556(4)	0.1479(2)	0.1737(1)	2.80(6)

(**Table** con'd.)

Atom	x	y	z	B_{eq} (Å^2)
C3A	0.2125(4)	0.3136(2)	0.1262(1)	2.99(7)
C4A	0.2537(4)	0.2561(2)	0.0762(1)	2.87(6)
C5A	0.3191(5)	0.0904(3)	0.0308(2)	3.84(8)
C6A	0.0934(4)	0.0964(3)	0.0720(2)	3.54(8)
C7A	0.3391(4)	0.1103(3)	0.2205(2)	3.51(8)
C8A	0.3057(4)	-0.0029(3)	0.2361(2)	3.53(8)
C9A	0.2765(5)	0.4251(3)	0.1238(2)	4.45(9)
C10A	0.0553(4)	0.3232(3)	0.1366(2)	3.49(8)
C11A	0.4029(7)	-0.1699(4)	0.2595(3)	8.9(2)
O1B	0.2585(3)	0.2319(2)	0.33758(9)	3.65(5)
O2B	0.1613(4)	0.5479(2)	0.2442(1)	4.86(7)
O3B	0.0742(3)	0.5376(2)	0.3243(1)	4.03(6)
O4B	0.0916(3)	0.0669(2)	0.4380(1)	4.10(6)
N1B	0.2739(3)	0.3398(2)	0.4377(10	2.83(6)
C1B	0.2860(4)	0.3949(3)	0.3857(1)	3.29(7)
C2B	0.2037(4)	0.3378(3)	0.3446(1)	3.13(7)
C3B	0.2533(4)	0.1651(3)	0.3836(1)	3.29(7)
C4B	0.3165(4)	0.2234(3)	0.4305(1)	3.13(7)
C5B	0.3756(5)	0.3914(3)	0.4750(2)	3.95(8)
C6B	0.1320(4)	0.3520(3)	0.4601(2)	3.56(8)
C7B	0.2186(5)	0.3948(3)	0.2922(1)	4.10(9)
C8B	0.1427(4)	0.5001(3)	0.2906(1)	3.24(8)
C9B	0.3507(6)	0.0704(3)	0.3724(2)	5.1(1)
C10B	0.1047(5)	0.1247(3)	0.3906(2)	3.72(8)
C11B	0.0913(6)	0.6495(3)	0.2366(2)	5.0(1)

Table A.2. Coordinates and isotropic thermal parameters for hydrogen atoms

Atom	x	y	z	B_{iso} (Å^2)
H4OA	-0.083(6)	0.322(4)	0.087(2)	10(2)
H1Aa	0.4090	0.1051	0.1229	3
H1Ab	0.2941	0.0174	0.1280	3
H2A	0.1589	0.1307	0.1766	3
H4Aa	0.1952	0.2825	0.0491	3
H4Ab	0.3483	0.2740	0.0688	3
H5Aa	0.4141	0.1128	0.0322	4
H5Ab	0.3148	0.0138	0.0313	4
H5Ac	0.2775	0.1164	-0.0003	4
H6Aa	0.0405	0.1228	0.1005	4
H6Ab	0.0541	0.1223	0.0404	4
H6Ac	0.0914	0.0197	0.0721	4
H7Aa	0.3191	0.1567	0.2489	4
H7Ab	0.4359	0.1145	0.2122	4
H9Aa	0.3741	0.4191	0.1173	5
H9Ab	0.2338	0.4649	0.0965	5
H9Ac	0.2618	0.4610	0.1559	5
H10Aa	0.0192	0.2545	0.1461	4
H10Aa	0.0400	0.3729	0.1641	4
H11Aa	0.4916	-0.1992	0.2682	11
H11Ab	0.3613	-0.2119	0.2327	11
H11Ac	0.3441	-0.1705	0.2892	11
H4OB	0.063(3)	0.007(2)	0.430(1)	1.9(6)
H1Ba	0.3817	0.3964	0.3756	4
H1Bb	0.2520	0.4666	0.3887	4
H2B	0.1085	0.3367	0.3553	4
H4Ba	0.2896	0.1849	0.4608	4
H4Bb	0.4154	0.2213	0.4270	4
H5Ba	0.4679	0.3848	0.4617	5

(**Table** con'd.)

Atom	x	y	z	B_{iso} (Å2)
H5Bb	0.3528	0.4655	0.4790	5
H5Bc	0.3698	0.3562	0.5077	5
H6Ba	0.0652	0.3201	0.4373	4
H6Bb	0.1282	0.3172	0.4929	4
H6Bc	0.1113	0.4264	0.4642	4
H7Ba	0.1819	0.3492	0.2658	5
H7Bb	0.3151	0.4076	0.2858	5
H9Ba	0.4436	0.0961	0.3680	6
H9Bb	0.3478	0.0211	0.4006	6
H9Bc	0.3212	0.0349	0.3415	6
H10Ba	0.0424	0.1844	0.3910	4
H10Bb	0.0813	0.0783	0.3625	4
H11Ba	0.1191	0.6797	0.2043	6
H11Bb	0.1155	0.6974	0.2640	6
H11Bc	-0.0071	0.6383	0.2365	6

APPENDIX B

CRYSTALLOGRAPHIC DATA FOR (2*R*,6*R*)-2-HYDROXYMETHYL-6-(METHOXYCARBONYL)METHYL-2,4,4-TRIMETHYLMORPHOLINIUM IODIDE

$C_{11}H_{22}NO_4I$, Mr = 359.2, orthorhombic, $P2_12_12_1$, a = 7.9611(4), b = 13.6603(5), c = 14.0588(9) Å, V = 1528.9(2) Å^3, Z = 4, D_x = 1.561 g cm^{-3} at 291 K, λ (Mo $K\alpha$) = 0.71073 Å, μ = 20.7 cm^{-1}, F(000) = 720, 6685 unique data measured, final R = 0.038 for 4863 reflections with I > 3σ(I).

Table B.1. Coordinates and isotropic thermal parameters

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij}\, a_i^* a_j^*\, \mathbf{a}_i \mathbf{a}_j$$

Atom	x	y	z	B_{eq} (Å^2)
I	0.72283(3)	0.19805(2)	0.31553(2)	4.409(4)
O1	0.3997(3)	0.3314(2)	0.1367(2)	3.02(4)
O2	0.7953(4)	0.5316(2)	0.0871(2)	4.66(5)
O3	0.5515(4)	0.5008(2)	0.0135(2)	4.56(6)
O4	0.3931(5)	0.1299(2)	0.1514(2)	5.14(7)
N	0.1985(3)	0.3646(2)	0.3058(2)	3.11(5)
C1	0.3680(4)	0.4099(2)	0.2904(2)	2.87(5)
C2	0.4101(4)	0.4228(2)	0.1850(2)	2.81(4)
C3	0.2380(4)	0.2864(2)	0.1415(2)	3.51(6)
C4	0.1844(5)	0.2738(2)	0.2448(3)	3.62(6)
C5	0.1853(6)	0.3332(3)	0.4074(3)	4.36(7)
C6	0.0603(5)	0.4365(3)	0.2885(3)	4.60(8)
C7	0.5891(4)	0.4576(2)	0.1780(2)	3.10(5)
C8	0.6372(5)	0.4982(2)	0.0828(3)	3.32(6)
C9	0.1120(6)	0.3362(4)	0.0771(3)	5.7(1)
C10	0.2695(6)	0.1829(3)	0.1018(3)	4.56(7)
C11	0.8611(7)	0.5730(3)	0.0005(3)	5.87(9)

Table B.2. Coordinates and isotropic thermal parameters for hydrogen atoms

Atom	x	y	z	B_{iso} (Å^2)
H4O	0.465(9)	0.169(4)	0.186(5)	11(2)
H1a	0.4506	0.3689	0.3168	3
H1b	0.3699	0.4722	0.3202	3
H2	0.3335	0.4678	0.1573	3
H4a	0.0704	0.2531	0.2453	4
H4b	0.2528	0.2243	0.2723	4
H5a	0.2715	0.2872	0.4211	5
H5b	0.1972	0.3886	0.4476	5
H5c	0.0788	0.3038	0.4179	5
H6a	0.0645	0.4582	0.2243	5
H6b	-0.0449	0.4061	0.3002	5
H6c	0.0734	0.4909	0.3298	5
H7a	0.6609	0.4038	0.1916	4
H7b	0.6054	0.5074	0.2242	4
H9a	0.1567	0.3405	0.0146	7
H9b	0.0109	0.2992	0.0758	7
H9c	0.0891	0.4001	0.1004	7
H10a	0.1671	0.1473	0.1047	5
H10b	0.3039	0.1888	0.0373	5
H11a	0.9784	0.5826	0.0058	7
H11b	0.8080	0.6341	-0.0112	7
H11c	0.8390	0.5297	-0.0509	7

APPENDIX C

CRYSTALLOGRAPHIC DATA FOR (2*R*,6*S*)-6-CARBOXYLATOMETHYL-2-HYDROXYMETHYL-2,4,4-TRIMETHYLMORPHOLINIUM

$C_{10}H_{19}NO_4 \bullet 2H_2O$, Mr = 253.3, monoclinic, $P2_1$, a = 7.8558(6), b = 9.8998(7), c = 8.6266(5) Å, β = 101.314(5)°, V = 657.9(2) Å^3, Z = 2, D_x = 1.278 g cm^{-3} at 294 K, λ (Cu $K\alpha$) = 1.54184 Å, μ = 8.45 cm^{-1}, F(000) = 276, 2641 unique data measured, final R = 0.0369 for 2571 reflections with I > 3σ(I).

Table C.1. Coordinates and isotropic thermal parameters

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij}\, a_i^* a_j^*\, \mathbf{a}_i \mathbf{a}_j$$

Atom	x	y	z	B_{eq} (Å^2)
O1	0.5734(1)	0	0.7318(1)	2.99(2)
O2	0.2195(2)	-0.2240(2)	0.5232(2)	4.03(3)
O3	0.4153(2)	-0.3195(2)	0.7085(2)	4.48(3)
O4	0.7828(2)	0.0209(2)	1.1530(1)	4.10(3)
N	0.9454(2)	-0.0086(2)	0.7484(2)	2.86(2)
C1	0.8128(2)	-0.0613(2)	0.6105(2)	2.90(3)
C2	0.6536(2)	-0.1108(2)	0.6667(2)	2.79(3)
C3	0.6790(2)	0.0589(2)	0.8696(2)	2.70(3)
C4	0.8600(2)	0.0943(2)	0.8384(2)	2.98(3)
C5	1.0877(2)	0.0633(2)	0.6876(2)	4.11(4)
C6	1.0276(2)	-0.1237(2)	0.8512(2)	3.71(3)
C7	0.5177(2)	-0.1685(2)	0.5331(2)	3.24(3)
C8	0.3719(2)	-0.2420(2)	0.5931(2)	2.92(3)
C9	0.5914(2)	0.1921(2)	0.8928(2)	3.26(3)
C10	0.6772(2)	-0.0322(2)	1.0131(2)	3.21(3)
O1W	0.2699(2)	0.1030(2)	0.0995(2)	4.77(3)
O2W	0.1097(2)	-0.0590(3)	0.2768(2)	9.42(5)

Table C.2. Coordinates and isotropic thermal parameters for hydrogen atoms

Atom	x	y	z	B_{iso} (Å^2)
H4O	0.713(4)	0.047(5)	1.218(4)	10(1)
H1a	0.875(2)	-0.133(2)	0.564(2)	3.1(4)
H1b	0.785(2)	0.018(2)	0.535(2)	3.1(4)
H2	0.687(2)	-0.179(2)	0.749(2)	2.6(3)
H4a	0.838(3)	0.170(2)	0.762(3)	4.3(5)
H4b	0.944(2)	0.104(2)	0.940(2)	3.1(4)
H5a	1.025(3)	0.132(3)	0.618(3)	5.8(6)
H5b	1.168(3)	0.095(2)	0.777(3)	4.5(5)
H5c	1.143(3)	-0.013(3)	0.626(3)	5.5(5)
H6a	0.932(2)	-0.183(2)	0.891(2)	3.7(4)
H6b	1.086(3)	-0.174(3)	0.786(3)	6.4(7)
H6c	1.109(3)	-0.084(3)	0.933(3)	5.4(5)
H7a	0.576(3)	-0.230(3)	0.482(3)	5.6(6)
H7b	0.471(3)	-0.104(2)	0.458(2)	4.2(5)
H9a	0.594(3)	0.259(3)	0.804(3)	5.7(6)
H9b	0.471(3)	0.177(3)	0.892(3)	4.9(5)
H9c	0.656(3)	0.227(3)	0.997(3)	4.8(5)
H10a	0.554(2)	-0.032(2)	1.030(2)	3.9(4)
H10b	0.725(3)	-0.125(2)	0.998(2)	3.5(4)
H1W1	0.373(3)	0.127(3)	0.159(3)	4.5(5)
H2W1	0.225(3)	0.052(3)	0.176(3)	5.5(6)
H1W2	0.139(5)	-0.088(5)	0.374(4)	11(1)
H2W2	-0.008(4)	-0.041(3)	0.244(3)	6.8(7)

APPENDIX D

CRYSTALLOGRAPHIC DATA FOR (2*R*,6*R*)-6-CARBOXYLATOMETHYL-2-HYDROXYMETHYL-2,4,4-TRIMETHYLMORPHOLINIUM

$C_{10}H_{19}NO_4 \bullet 2H_2O$, Mr = 253.3, orthorhombic, $P2_12_12_1$, a = 7.8402(7), b = 12.5209(6), c = 13.0286(7) Å, V = 1279.0(3) Å^3, Z = 4, D_x = 1.316 g cm^{-3} at 292 K, λ (Cu $K\alpha$) = 1.54184 Å, μ = 8.7 cm^{-1}, $F(000)$ = 552, 2597 unique data measured, final R = 0.0334 for 2435 reflections with I > 3σ(I).

Table D.1. Coordinates and isotropic thermal parameters

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij}\, a_i^* a_j^*\, \mathbf{a}_i \mathbf{a}_j$$

Atom	x	y	z	B_{eq} (Å^2)
O1	0.3308(1)	0.55093(7)	0.59485(7)	2.04(2)
O2	0.1842(2)	0.7990(1)	0.77761(9)	4.10(2)
O3	0.3618(2)	0.79070(9)	0.6447(1)	3.78(2)
O4	0.6578(1)	0.46173(9)	0.62969(9)	3.35(2)
N	0.1162(2)	0.46807(9)	0.43284(9)	2.45(2)
C1	0.0530(2)	0.5417(1)	0.5158(1)	2.35(2)
C2	0.1918(2)	0.6138(1)	0.5571(1)	1.94(2)
C3	0.4099(2)	0.4834(1)	0.5193(1)	2.06(2)
C4	0.2752(2)	0.4118(1)	0.4696(1)	2.36(2)
C5	0.1424(2)	0.5273(1)	0.3338(1)	3.46(3)
C6	-0.0186(2)	0.3848(1)	0.4143(2)	4.03(3)
C7	0.1185(2)	0.6755(1)	0.6469(1)	2.18(2)
C8	0.2329(2)	0.7618(1)	0.6923(1)	2.45(2)
C9	0.5197(2)	0.5478(1)	0.4445(1)	2.90(3)
C10	0.5232(2)	0.4066(1)	0.5803(1)	2.45(2)
O1W	0.6750(2)	0.6867(1)	0.6705(1)	4.46(3)
O2W	0.8622(2)	0.7462(1)	0.8474(1)	5.35(3)

Table D.2. Coordinates and isotropic thermal parameters for hydrogen atoms

Atom	x	y	z	B_{iso} (Å^2)
H4O	0.705(4)	0.414(2)	0.659(2)	6.0(6)
H1a	0.014(2)	0.493(1)	0.571(1)	2.8(3))
H1b	-0.042(2)	0.584(1)	0.485(1)	2.4(3)
H2	0.226(2)	0.660(1)	0.503(1)	2.3(3)
H4a	0.314(3)	0.374(1)	0.412(1)	3.3(4)
H4b	0.240(2)	0.357(1)	0.522(1)	2.9(3)
H5a	0.215(3)	0.587(2)	0.346(1)	4.2(4)
H5b	0.170(3)	0.475(2)	0.283(2)	4.9(5)
H5c	0.028(3)	0.555(2)	0.312(2)	6.1(6)
H6a	-0.038(3)	0.344(2)	0.487(1)	4.4(4)
H6b	-0.115(3)	0.425(2)	0.389(2)	5.8(6)
H6c	0.023(3)	0.342(2)	0.360(2)	5.6(5)
H7a	0.092(2)	0.628(1)	0.700(1)	2.4(3)
H7b	0.016(2)	0.710(1)	0.624(1)	3.2(4)
H9a	0.615(3)	0.584(2)	0.476(2)	5.5(5)
H9b	0.557(3)	0.499(1)	0.394(2)	5.2(5)
H9c	0.455(3)	0.610(1)	0.405(1)	4.7(5)
H10a	0.454(2)	0.367(1)	0.632(1)	3.0(4)
H10b	0.561(3)	0.353(1)	0.535(1)	3.6(4)
H1W1	0.596(4)	0.723(2)	0.663(2)	6.9(7)
H2W1	0.677(4)	0.620(2)	0.659(2)	7.4(7)
H1W2	0.949(4)	0.781(2)	0.820(2)	6.3(6)
H2W2	0.820(4)	0.734(2)	0.791(2)	8.7(8)

APPENDIX E

CRYSTALLOGRAPHIC DATA FOR METHYL (*2S,6S;2R,6R*)-2-[6-(2-CYANOETHYL)-4,6-DIMETHYLMORPHOLINYL]ACETATE

$C_{12}H_{20}N_2O_3$, Mr = 240.3, triclinic, $P\bar{1}$, a = 5.6057(4), b = 9.1737(7), c = 14.1151(10) Å, α = 74.430(7), β = 88.908(6), γ = 79.302(6)°, V = 686.7(1) Å^3, Z = 2, D_x = 1.162 g cm^{-3} at 297 K, λ (Mo $K\alpha$) = 0.71073 Å, μ = 0.8 cm^{-1}, $F(000)$ = 260.0, 2409 unique data measured, final R = 0.040 for 1850 reflections with $I > 2\sigma(I)$.

Table E.1. Coordinates and isotropic thermal parameters

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j$$

Atom	x	y	z	B_{eq} (Å^2)
O1	0.1478(2)	0.3437(1)	0.77508(6)	3.84(2)
O2	0.0572(2)	0.6770(1)	0.5993(1)	6.85(4)
O3	0.4592(2)	0.6267(1)	0.59987(8)	5.33(3)
N1	-0.1319(3)	0.1366(1)	0.7412(1)	5.00(3)
N2	-0.6392(4)	0.7041(2)	0.8732(1)	8.44(5)
C1	0.0433(3)	0.1943(2)	0.6707(1)	4.97(4)
C2	0.0703(3)	0.3509(2)	0.6780(1)	3.82(3)
C3	-0.0015(3)	0.2750(2)	0.8525(1)	4.04(4)
C4	-0.0398(3)	0.1219(2)	0.8395(1)	5.19(4)
C5	-0.1843(4)	-0.0096(2)	0.7321(2)	7.71(6)
C6	0.2589(3)	0.4188(2)	0.6118(1)	4.51(4)
C7	0.2421(3)	0.5856(2)	0.6039(1)	4.34(4)
C8	0.4578(4)	0.7879(2)	0.5869(2)	6.75(5)
C9	0.1474(4)	0.2508(2)	0.9463(1)	5.76(5)
C10	-0.2458(3)	0.3815(2)	0.8519(1)	4.33(4)
C11	-0.2261(3)	0.5475(2)	0.8417(1)	5.43(4)
C12	-0.4585(4)	0.6373(2)	0.8589(1)	5.78(5)

Table E.2. Coordinates and isotropic thermal parameters for hydrogen atoms

Atom	x	y	z	B_{iso} (Å^2)
H1a	-0.007(3)	0.200(2)	0.606(1)	5.5(4)
H1b	0.201(3)	0.124(2)	0.683(1)	5.9(4)
H2	-0.087(2)	0.420(1)	0.6595(9)	4.2(3)
H4a	0.118(3)	0.046(2)	0.854(1)	6.6(4)
H4b	-0.151(3)	0.081(2)	0.889(1)	6.5(4)
H5a	-0.301(4)	-0.046(2)	0.783(1)	10.2(6)
H5b	-0.242(4)	0.006(2)	0.663(1)	10.3(6)
H5c	-0.036(3)	-0.093(2)	0.748(1)	8.9(5)
H6a	0.229(3)	0.414(2)	0.547(1)	5.4(4)
H6b	0.422(3)	0.361(2)	0.636(1)	5.9(4)
H8a	0.386(4)	0.819(2)	0.644(1)	10.3(6)
H8b	0.369(4)	0.855(2)	0.525(1)	10.8(6)
H8c	0.615(4)	0.802(2)	0.587(2)	11.3(6)
H9a	0.053(3)	0.214(2)	1.002(1)	7.7(5)
H9b	0.299(3)	0.173(2)	0.945(1)	7.9(5)
H9c	0.187(3)	0.354(2)	0.949(1)	7.9(5)
H10a	-0.322(3)	0.340(2)	0.913(1)	6.0(4)
H10b	-0.346(2)	0.381(1)	0.7991(9)	4.1(3)
H11a	-0.176(3)	0.597(2)	0.776(1)	8.7(5)
H11b	-0.118(3)	0.554(2)	0.890(1)	7.2(4)

VITA

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Candidate: Guobin Sun

Major Field: Chemistry

Title of Dissertation: Reaction-intermediate Analogues: Design, Syntheses, and Inhibitory Studies with Carnitine Acetyltransferase

Approved:

Richard D. Gandour
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Date of Examination:

June 20, 1994